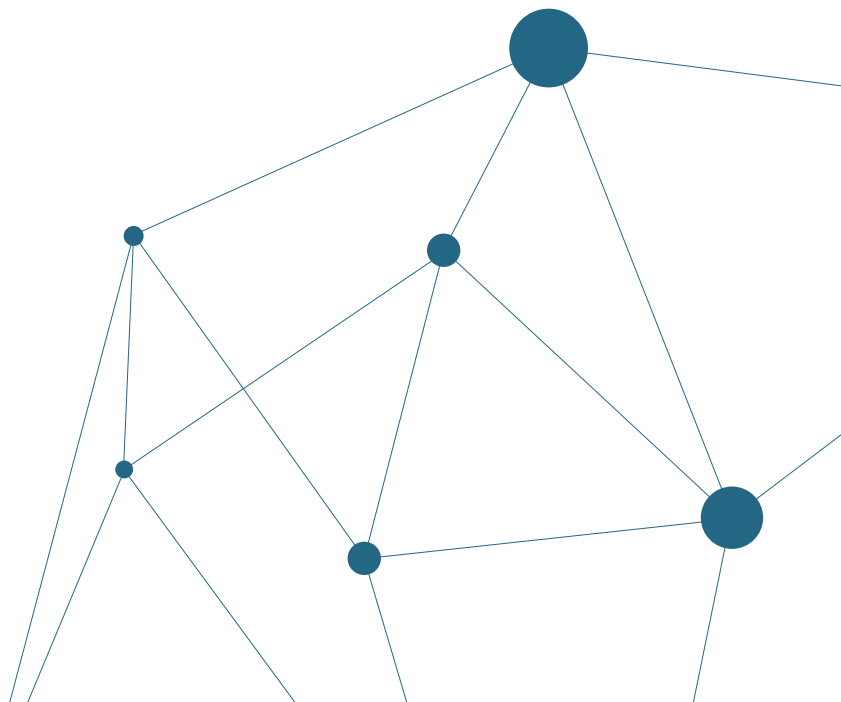


G

Gene Expression Analysis

01. qPCR Array Service

02. qPCR Array System



01. qPCR Array Service

<i>AccuPower</i> [®] qPCR Array Service	251
<i>AccuPower</i> [®] qPCR Array Service FAQs	255

Description

Having rich experiences and highly advanced infrastructure, BIONEER provides accurate and reliable results quickly by following the MIQE (Minimum Information for Publication of Quantitative Real-Time PCR Experiments) guideline.

Features and Benefits

Quick & Accurate

Bioneer’s gene expression analysis service provides high-quality data by following the *MIQE Guidelines. Accurate results, verified by numerous researches done by our customers, can be quickly gained and readily presented to your paper.

*The Minimum Information for Publication of Quantitative Real-Time PCR Experiments (MIQE) “qPCR gene expression analysis” Agreed for quality control of papers: Bustin, S.A., et al. 2009. The MIQE Guidelines: Minimum Information for Publication of Quantitative Real-Time PCR Experiments, Clinical Chemistry 55:4, 611-622.

Save time and money

Precise primer designs, synthesis, and tests are crucial for accurate Real-Time PCR data. Even for experienced researchers, designing primers require a considerable amount of time and money, as those require optimized designs and numerous validation tests. Over the last ten years, our Gene Expression Assay Team has done continuous tests that allowed us to acquire over 12,000 pairs of proven primer sets, having favorable specificity and sensitivity for getting high-quality data. Furthermore, our proven experts, having years of experience, can build and test the best primers to quickly provide the gene synthesis analysis data, no matter what kind of gene analysis service orders we receive. For improved accuracy, we do primer tests to test the target-specific amplification of primers. If the results do not meet our quality standards, we will start the whole process again from the beginning.

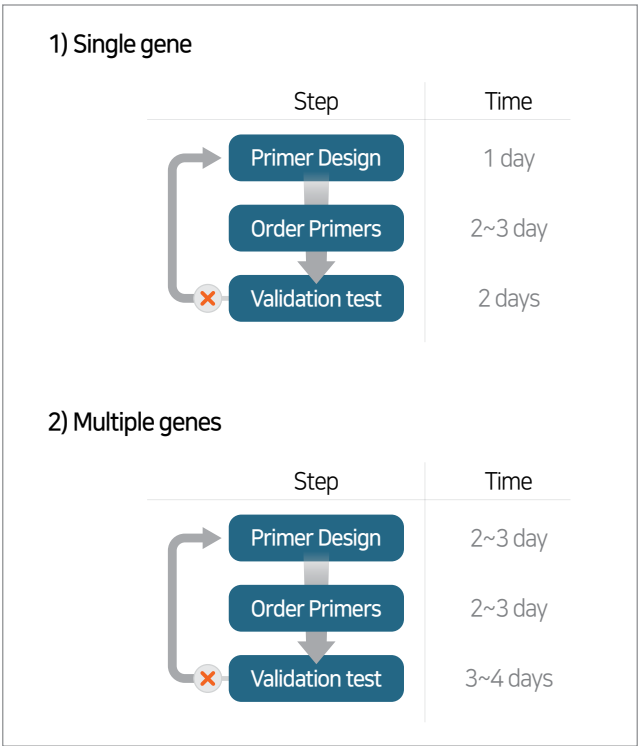


Figure 1. Time required for primer synthesis and verification

We test our primer sets to check their target-specific amplification for our customer’s accurate qPCR results. If the primer sets fail the validation tests, we will start the whole process again from the very beginning. 1) A Single target gene: Takes minimum of 4 business days, 2) Multiple target genes: Takes 4-7 business days; validation tests on primer sets are performed to check the target-specific amplification.

Service Information

1. Service WorkFlow

Depending on the type of requested samples (Cell or Tissue / Total RNA / cDNA), services may be done differently.

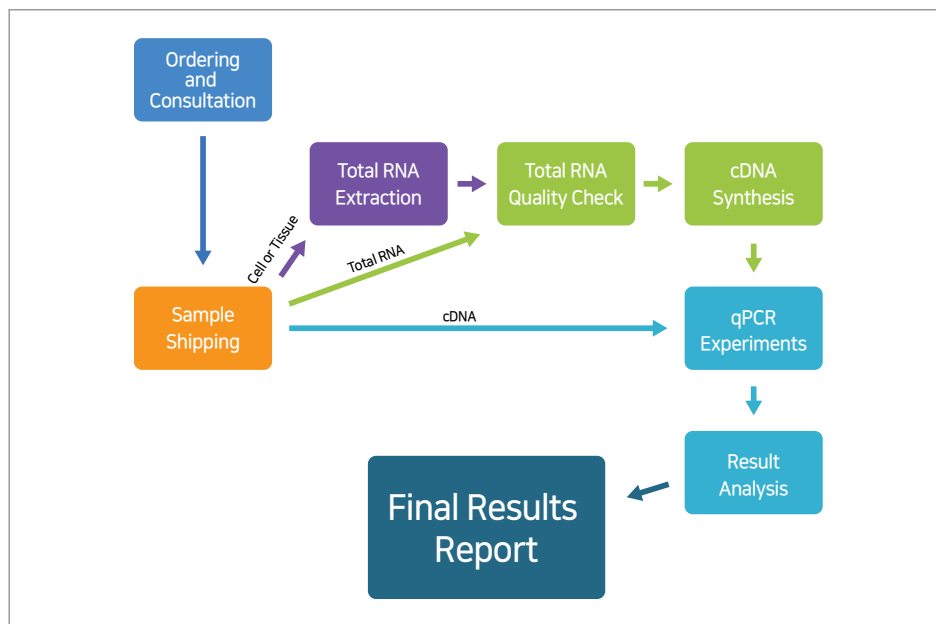


Figure 2. qPCR Array Service work flow. Process for service based on different sample type.

1) Ordering and Consultation

Complete the "qPCR Array Service Order Form" from our homepage (www.bioneer.com). Then, send an email to the Gene Expression Analysis Team (qPCRarray@bioneer.com) on the Gene Expression Analysis page so that we will guide you to the next step.

2) Sample Shipment

Depending on the sample types that you are shipping, please follow the instructions for the sample preparations below. There are many types of shipping methods depending on the types of samples that you wish to send. If you have any further questions, feel free to contact us at qPCRarray@bioneer.com.

3) Total RNA Extraction and gDNA Removal

After the samples arrive, we must first choose the suitable RNA extraction kit depending on their types. This is to ensure the total RNA extraction is done with the best quality and purity. Furthermore, we treat the isolated RNA with RNase free DNase I to remove the gDNAs that can affect the mRNA quantification.

4) Quality check

All the impurities in the extracted total RNA may affect the amplification efficiency by inhibiting PCR polymerase reaction. Therefore, spectrophotometers are used for measuring both the quantity and purity of nucleic acids. Since it is difficult to accurately quantify the amount of degraded RNA, we measure the RNA quality score (RQS) with capillary gel electrophoresis. All those measurements are recorded in the report and sent to the customers. We will proceed to the next step only if the purity and the degree of degradation of the RNA satisfy our standards (RQS > 8.0).

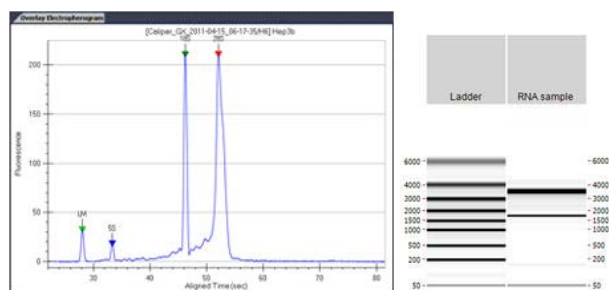


Figure 3. Example of RNA quality check (left) Caliper LabChip™ GX results (right)

5) cDNA synthesis

cDNA synthesis is one of the most crucial processes along with the RNA quality tests, as it can severely affect the gene synthesis analysis results. Our patented thermostable RTase (*RocketScript™*) and CTRT (Cyclic Temperature Reverse Transcription) technologies allow us to synthesis full-length cDNAs even with RNAs having complicated secondary structures, which can help us to have accurate and precise expression analysis results. We use oligo dT or random primers (dN₆, dN₁₂) depending on the customer's research purposes.

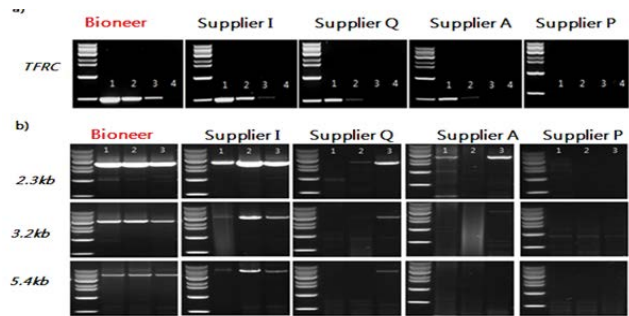


Figure 4. Comparison of cDNA synthesis efficiency with Bioneer.

6) qPCR

There are many experts in BIONEER that can accurately perform qPCR for this service. We use *Exicycler™* 96 Real-Time Quantitative Thermal Cycler, having an exceptional sensitivity with our own patented optical detection technology. All our products and services, including the instruments used in *AccuPower®* qPCR Array Service, are regularly inspected and managed under ISO regulations.

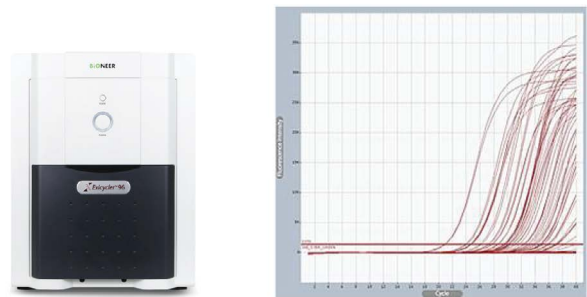


Figure 5. (1) *Exicycler™* 96 Real-Time Quantitative Thermal Block
(2) Amplification Curve

7) Analyzing and providing results

2^{-ΔΔCt} method will be used for the relative quantitative data analysis from the qPCR results. We will send an Excel file containing the qPCR data. The analysis results will also contain raw Ct values, melting curve analysis, *p*-value, fold change, scatter plots, volcano plots, and heat map image. If you have our *Exicycler™* analysis software, we can also send the qPCR raw data. Upon customers' requests, we can also provide Information on primers such as genetic information, amplicon size, and PCR conditions under the MIQE guidelines.

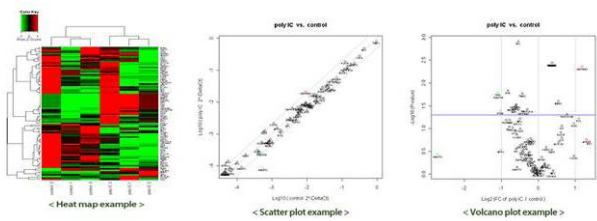


Figure 6. qPCR analysis results

2. Service Duration

Number of analyzed genes	Service Period
≤ 16 genes	2 weeks
≤ 32 genes	2~3 weeks
≤ 96 genes	3~5 weeks

* Additional time may be required for service requests using primers not owned by the company.

○ Sample preparation for qPCR Array Service

1. cDNA Sample

- Required cDNA volume (for triplication basis)

Number of analyzed genes	cDNA volume
≤ 16 genes	200 µl
≤ 32 genes	400 µl
≤ 96 genes	900 µl

*Depending on your experimental design, we may ask for more details.

- Precautions for sample preparation**

- Synthesize cDNA using high purity RNA as shown below.
 - Spectrophotometer 260/280 nm value of 1.8 or higher
 - RNA Electrophoresis 28S / 18S rRNA band 2 : 1 ratio
- Check whether the recommended amount of RNA used for cDNA synthesis is 0.4 ~ 1 µg / 20 µl (1 rxn).
 - Genes with low expression levels can only be synthesized with higher RNA content for more accurate results.
- Store synthesized cDNA samples below -20 °C.

2. Total RNA Samples

- Required total RNA concentration (for triplication basis)

Number of analyzed genes	Minimum volume	Minimum concentration	Total RNA
≤ 16 genes	15 µl	100 ng/µl	> 5 µg
≤ 32 genes			> 10 µg
≤ 96 genes			> 20 µg

*Depending on your experimental design, we may ask for more details.

- Precautions for sample preparation**

- Undergo RNA extraction quickly in an RNase-free environment.
- During the extraction process, remove genomic DNA by DNase I treatment.
- Check whether the 260 / 280 nm value of the RNA extracted from the spectrophotometer is 1.8 or more.
- Test whether the ratio of RNA Electrophoresis 28S / 18S rRNA band of extracted RNA is 2: 1.
- Store the extracted RNA samples at -80 °C.

3. Request of RNA Extraction

- Tissue sample**

- Required sample amount : 25~50 mg
- How to prepare samples
After tissue extraction, to minimize the RNA degradation, select one of the following methods to prepare the samples.

Method 1. Freeze the tissue samples in a liquid nitrogen and store at -80 °C.

Method 2. Treat them with a RNA stabilization solution according to its manual and store at -80 °C.

Method 3. Treat them using lysis buffer (Guanidinium thiocyanate buffer*) containing 1% beta-mercaptoethanol in the ratio of 500 µl per 20~30 mg of tissue. Then, disintegrate using a homogenizer and store at -80°C. (*AccuPrep® RB buffer of Universal RNA Extraction Kit)

- Cell sample**

- Required sample amount: 10⁴~10⁸ cells
- How to prepare samples
After cell harvesting, to minimize the RNA degradation, select one of the following methods to prepare the samples.

Method 1. Rapidly freeze the samples Immediately with liquid nitrogen and store at -80 °C.

Method 2. Treat them with a RNA stabilization solution according to its manual and store at -80 °C.

Method 3. Treat them with lysis buffer (Guanidinium thiocyanate buffer*) containing 1 % Beta-mercaptoethanol.
- Add 400 µl of lysis buffer per 10⁴ ~ 10⁶ of cells.
- Disintegrate using a homogenizer and store at -80 °C. (* RB buffer of AccuPrep® Universal RNA Extraction Kit)

- Blood and other samples**

We may need to have a separate discussion for samples other than tissues and cells. For blood samples, we highly recommend using EDTA-tube.

- For other samples including the blood, please contact us at qPCRarray@bioneer.com.

○ Technical Support

- Address: 8-11, Munpyeongseoro, Daedeok-gu, Daejeon 34302, Republic of Korea Bioneer Gene Expression Analysis Team
- E-mail: qPCRarray@bioneer.com
(GMT+9 09:00 to 18:00; Monday to Friday)

○ Ordering Information

Cat. No.	Service Description
S-6040-RE	AccuPower® qPCR Array Service: Total RNA Extraction
S-6040-DT	AccuPower® qPCR Array Service: DNase I Treatment
S-6040-RQ	AccuPower® qPCR Array Service: Total RNA Quality Check
S-6040-CS	AccuPower® qPCR Array Service: cDNA Synthesis
S-6040-QA	AccuPower® qPCR Array Service: qPCR Analysis

1. What is qPCR Array Service?

qPCR Array Service provides accurate quantitative results for many genes by providing a combination of quantitative PCR with high sensitivity and reliability for gene expression analysis and microarray technique for analyzing expression patterns of several genes at once.

2. How can I place an order?

Please refer to Gene Expression Service Ordering Protocol tab of Bioneer website(www.bioneer.com), fill out the qPCR Array Service Order Form, and send it to qPCRarray@bioneer.com.

3. How is the final report provided?

1) qPCR array service report (PDF file)

Provides workflow-specific results and fold change values for the entire qPCR Array Service.

2) Fold change analysis file (Excel file)

Provides the calculation sheet recorded with the analyzed fold change values such as raw Ct, dCt, $2^{-\Delta\Delta Ct}$, fold change, etc., applied with a relative quantification $2^{-\Delta\Delta Ct}$ method.

3) qPCR array service raw data (Excel file)

Provides qPCR results with the analyzed raw data such as PCR protocol, Ct, Ct Threshold, Baseline, Tm values, etc., through *Exicycler* analysis program.

4) qPCR array service result interpretation help (PDF file)

Provides guidelines on qPCR array service and result analysis.

4. Do I need to prepare an assay primer separately along with the samples for this service?

You don't need to. As long as you provide an accurate information for the samples, we will undergo the analysis after the primer design and validation steps.

5. DNase I cannot be treated in our laboratory. What should I do?

We also an additional service called "DNase I treatment". Although we only apply the service for those requesting the RNA sample analysis, it is provided as default when requesting qPCR array services from the RNA extraction.

6. How can I identify the Gene ID or accession number of the target gene?

1) Check your Gene ID

(1) Search by target gene symbol in NCBI Gene (<https://www.ncbi.nlm.nih.gov/gene/>).

(2) Gene ID is the number found at the bottom of the gene name. Check the gene name and description to make sure it is correct.

2) Find Accession number

(1) Search by target gene symbol in NCBI Gene (<https://www.ncbi.nlm.nih.gov/gene/>).

(2) After selecting the gene name, under 'mRNA and Protein(s)' category, you can see the mRNA and protein accession number in the category.

7. How do I select a reference gene (internal control/house-keeping gene) suitable for my experiment?

The relative analysis $2^{-\Delta\Delta Ct}$ method uses a reference gene to normalize the extraction yield, reverse transcription yield, amplification efficiency, and the RNA quantity. The selected gene must be able to undergo stable expression and closely related to the total RNA contents of the samples. Also, as selecting the reference genes are highly important to get accurate qPCR results for each experiment, we advise choosing them based on the related papers and experiment results. If you find this step difficult, we provide an additional service for reference gene screening. Refer to 'AccuPower® qPCR Array System: Reference qPCR primer Set' page.

02. qPCR Array System

AccuPower® qPCR Array System: qPCR Array Panel	
AccuPower® qPCR Array System: Customized qPCR Panel kit	257
AccuPower® qPCR Array System: Human Cancer qPCR Panel kit	259
AccuPower® qPCR Array System: Immune qPCR Panel kit	263
AccuPower® qPCR Array System: Immune checkpoint qPCR Panel kit	270
AccuPower® qPCR Array System: qPCR Array Primer/Probe Set	
AccuPower® qPCR Array System: Reference qPCR primer set	273
AccuPower® qPCR Array System: Human 5-plex Reference qPCR Primer and Probe Set ..	275
Single Gene qPCR Primer Set	277

AccuPower® qPCR Array System: Customized qPCR Panel kit

○ Description

By using 12,000 proven primer pairs, we provide customized panels that are tailored to the purpose of the experimenter. We can produce various panel pathway and disease related genes in the form of panel which you want to screen at once. You can easily analyze target gene expression patterns by using Customized qPCR panel kit. Also, simply mix with cDNA samples and AccuPower® 2X GreenStar™ Master Mix to start your analysis as the primer pairs are already coated.

○ Features and Benefits

1. Accurate

Shows high specificity and efficacy by using the panels with the primers meeting the MIQE (Minimum Information for Publication of Quantitative Real-Time PCR Experiments) guidelines^[1] to show accurate qPCR data (Figure 4).

2. Fast & Economical

Save valuable time and money for making and synthesizing primers for analysis and verifying them.

3. Convenient

Perform the qPCR analysis only by using the cDNA samples and AccuPower® 2X GreenStar™ Master Mix.

4. Customizable

Customize the panels for various purposes such as researching disease mechanism and gene functions, screening for new drug, and developing health functional foods. If you find configuring the panels difficult, please refer to classification in the qPCR array library, provided separately.

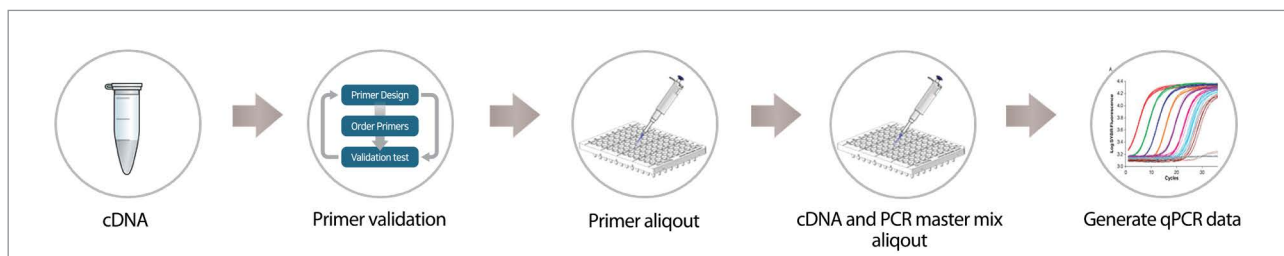


Figure 1. General process of qPCR.

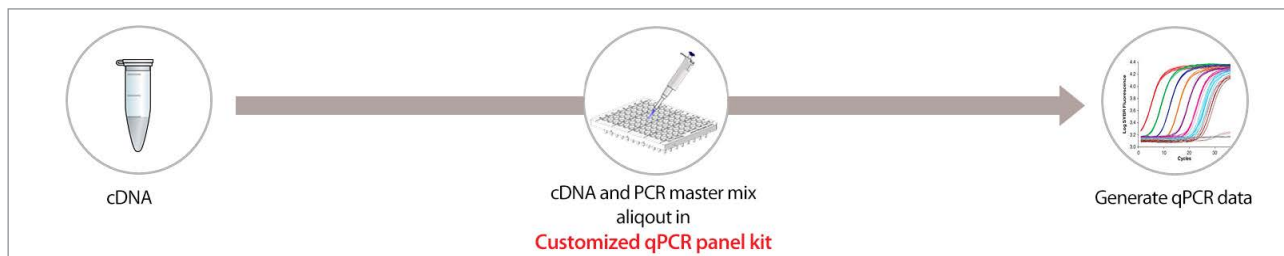


Figure 2. Process of qPCR with customized qPCR panel.

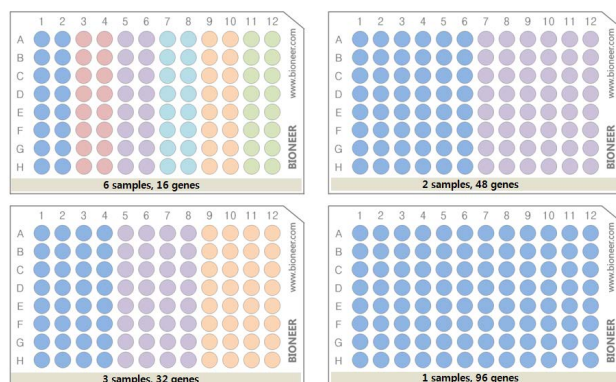


Figure 3. Example of customized qPCR panel layout

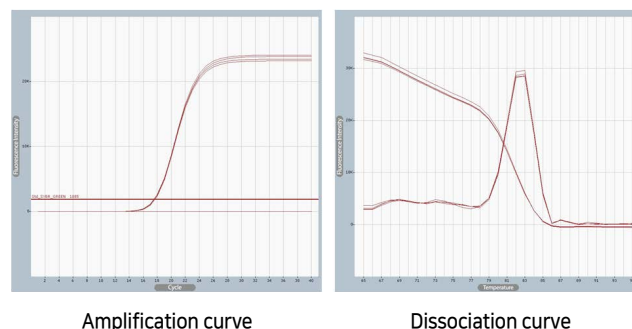


Figure 4. Validated specific primer sets. (example. Human ACTB gene).

AccuPower® qPCR Array System: Customized qPCR Panel kit

Ordering Information

Cat. No	Product Description
S-6041	AccuPower® qPCR Array System: Customized qPCR Panel kit

*Species other than Human, Mouse, and Rat require samples for primer verification.

*AccuPower® 2X GreenStar™ Master Mix is sold separately.

qPCR array library categories

- | | |
|------------------------------------------|---------------------------------|
| 1. Cancer | 6. Signal Transduction |
| 2. Cellular Differentiation & Regulation | 7. Stem Cell & Cell Development |
| 3. Disease | 8. Toxicology & Drug Metabolism |
| 4. Immunology | 9. Biological Process |
| 5. Metabolism | 10. Unknown Genes |

*Panel can be composed of the other genes you want.

How to order

1. Download the order form on the homepage.
(Order form address : <https://eng.bioneer.com/20-s-6041.html>)
2. Please fill out the details on the order form.
3. If you have a panel layout you want, please complete it in the 4. plate map.
4. Send the completed order form to the Gene Expression Analysis Team mail (qPCR@bioneer.com).
5. Based on the information on the order form, we will e-mail you the genetic information, final quotes and panel production based on quantity.
6. Once you receive the reminder e-mail, decide whether to proceed with the final service and let us know via e-mail, the service will be started.

Technical Support

- Address: 8-11, Munpyeongseoro, Daedeok-gu, Daejeon 34302, Republic of Korea Bioneer Gene Expression Analysis Team
- E-mail: qPCRarray@bioneer.com (GMT+9 09:00 to 18:00; Monday to Friday).

Reference

[1] Bustin, S.A., et al. 2009. The MIQE Guidelines: Minimum Information for Publication of Quantitative Real-Time PCR Experiments. *Clinical Chemistry* 55:4, 611-622

AccuPower® qPCR Array System: Human Cancer qPCR Panel kit

○ Description

Human Cancer qPCR Panel Kit can analyze various cancer pathway related genes at once.

The constituent primers in this panel are designed to minimize non-specific reactions through validation experiments and to show high sensitivity and reproducibility for the target gene.

You can easily perform qPCR analysis of cancer-related genes with cDNA samples and AccuPower® 2X GreenStar™ Master Mix distributions on a panel coated with a proven primer set.

Get reliable results easily with the Human Cancer qPCR panel.

○ Features and Benefits

1. Accurate

Shows high specificity and efficacy by using the panels with the primers meeting the MIQE (Minimum Information for Publication of Quantitative Real-Time PCR Experiments) guidelines^[1] to show accurate qPCR data (Figure 1).

2. Fast & economical

Save valuable time and money for making and synthesizing primers for analysis and verifying them.

3. Convenient

Perform the qPCR analysis only by using the cDNA samples and AccuPower® 2X GreenStar™ Master Mix.

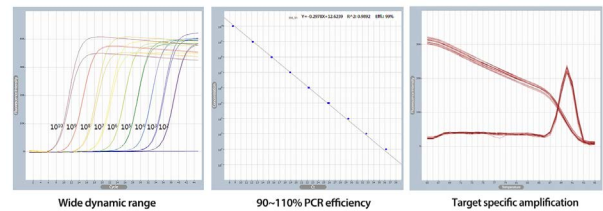


Figure 1. Validated primer data: wide dynamic range, one peak dissociation curve & PCR efficiency (Example. AKT gene).

○ Ordering Information

Cat. No	Product Description
S-6042-PH1	AccuPower® qPCR Array System: Human Cancer qPCR Panel kit

■ Plate Map

It is consisted of 88 cancer-related genes, 5 reference genes, and 3 control wells. Cancer-related genes were selected like below (Figure 2).

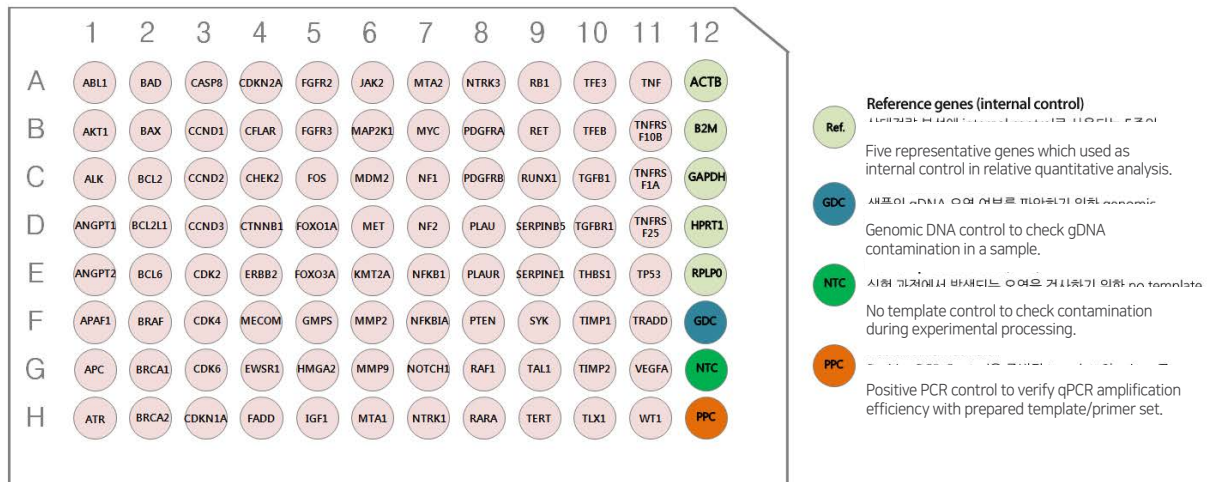


Figure 2. Layout of Human Cancer qPCR Panel.

AccuPower® qPCR Array System: Human Cancer qPCR Panel kit

■ Gene List

Well	Gene symbol	Description	Accession #
A01	ABL1	ABL proto-oncogene 1, non-receptor tyrosine kinase	NM_005157, NM_007313
B01	AKT1	v-akt murine thymoma viral oncogene homolog 1	NM_001014431, NM_001014432, NM_005163
C01	ALK	anaplastic lymphoma receptor tyrosine kinase	NM_004304
D01	ANGPT1	angiotensinogen 1	NM_001146, NM_001199859
E01	ANGPT2	angiotensinogen 2	NM_001118887, NM_001118888, NM_001147
F01	APAF1	apoptotic peptidase activating factor 1	NM_001160, NM_013229, NM_181861, NM_181868, NM_181869
G01	APC	adenomatous polyposis coli	NM_000038.5, NM_001127510, NM_001127511
H01	ATR	ATR serine/threonine kinase	NM_001184
A02	BAD	BCL2-associated agonist of cell death	NM_004322, NM_032989
B02	BAX	BCL2-associated X protein	NM_001291428, NM_001291429, NM_001291430, NM_001291431, NM_004324, NM_138761, NM_138763, NM_138764
C02	BCL2	B-cell CLL/lymphoma 2	NM_000633, NM_000657
D02	BCL2L1	BCL2-like 1	NM_001191, NM_138578
E02	BCL6	B-cell CLL/lymphoma 6	NM_001130845, NM_001134738, NM_001706
F02	BRAF	B-Raf proto-oncogene, serine/threonine kinase	NM_004333
G02	BRCA1	breast cancer 1, early onset	NM_007294, NM_007297, NM_007298, NM_007299, NM_007300
H02	BRCA2	breast cancer 2, early onset	NM_000059
A03	CASP8	caspase 8, apoptosis-related cysteine peptidase	NM_001080124, NM_001080125, NM_001228, NM_033355, NM_033356, NM_033358
B03	CCND1	cyclin D1	NM_053056
C03	CCND2	cyclin D2	NM_001759
D03	CCND3	cyclin D3	NM_001136017, NM_001136125, NM_001136126, NM_001287427, NM_001287434, NM_001760
E03	CDK2	cyclin-dependent kinase 2	NM_001290230, NM_001798, NM_052827
F03	CDK4	cyclin-dependent kinase 4	NM_000075
G03	CDK6	cyclin-dependent kinase 6	NM_001145306, NM_001259
H03	CDKN1A	cyclin-dependent kinase inhibitor 1A (p21, Cip1)	NM_000389, NM_001220777, NM_001220778, NM_001291549, NM_078467
A04	CDKN2A	cyclin-dependent kinase inhibitor 2A	NM_000077, NM_001195132, NM_058195, NM_058197
B04	CFLAR	CASP8 and FADD-like apoptosis regulator	NM_001127183, NM_001127184, NM_001202515, NM_001202516, NM_001202517, NM_001202518, NM_001202519, NM_003879
C04	CHEK2	checkpoint kinase 2	NM_001005735, NM_001257387, NM_007194, NM_145862,
D04	CTNNB1	catenin (cadherin-associated protein), beta 1, 88kDa	NM_001098209, NM_001098210, NM_001904
E04	ERBB2	erb-b2 receptor tyrosine kinase	NM_001005862, NM_001289936, NM_001289937, NM_001289938, NM_004448
F04	MECOM	MDS1 and EVI1 complex locus	NM_001105077, NM_001105078, NM_001163999, NM_001164000, NM_001205194, NM_004991, NM_005241
G04	EWSR1	EWS RNA-binding protein 1	NM_001163285, NM_001163286, NM_001163287, NM_005243, NM_013986
H04	FADD	Fas (TNFRSF6)-associated via death domain	NM_003824.3

AccuPower® qPCR Array System: Human Cancer qPCR Panel kit

Well	Gene symbol	Description	Accession #
A05	FGFR2	fibroblast growth factor receptor 2	NM_000141, NM_001144913, NM_001144914, NM_001144915, NM_001144916, NM_001144917, NM_001144918, NM_001144919, NM_022970, NM_023029
B05	FGFR3	fibroblast growth factor receptor 3	NM_000142, NM_001163213, NM_022965
C05	FOS	FBJ murine osteosarcoma viral oncogene homolog	NM_005252
D05	FOXO1	forkhead box O1	NM_002015.3
E05	FOXO3	AF6q21, FKHL1, FKHL1P2, FOXO2A, FOXO3	NM_001455, NM_201559
F05	GMPS	guanine monphosphate synthase	NM_003875
G05	HMGA2	high mobility group AT-hook 2	NM_001300918, NM_001300919, NM_003483, NM_003484
H05	IGF1	insulin-like growth factor 1	NM_000618, NM_001111283, NM_001111284, NM_001111285
A06	JAK2	Janus kinase 2	NM_004972
B06	MAP2K1	mitogen-activated protein kinase kinase 1	NM_002755
C06	MDM2	MDM2 proto-oncogene, E3 ubiquitin protein ligase	NM_001145337, NM_001145339, NM_001145340, NM_001278462, NM_002392
D06	MET	MET proto-oncogene, receptor tyrosine kinase	NM_000245, NM_001127500
E06	KMT2A	lysine (K)-specific methyltransferase 2A	NM_001197104, NM_005933
F06	MMP2	matrix metalloproteinase 2 (gelatinase A, 72kDa gelatinase, 72kDa type IV collagenase)	NM_001127891, NM_001302508, NM_001302509, NM_001302510, NM_004530
G06	MMP9	matrix metalloproteinase 9 (gelatinase B, 92kDa gelatinase, 92kDa type IV collagenase)	NM_004994
H06	MTA1	metastasis associated 1	NM_001203258, NM_004689
A07	MTA2	metastasis associated 1 family, member 2	NM_004739
B07	MYC	v-myc avian myelocytomatosis viral oncogene homolog	NM_002467
C07	NF1	neurofibromin 1	NM_000267, NM_001042492, NM_001128147
D07	NF2	neurofibromin 2 (merlin)	NM_000268, NM_016418, NM_181825, NM_181828, NM_181829, NM_181830, NM_181831, NM_181832, NM_181833
E07	NFKB1	nuclear factor of kappa light polypeptide gene enhancer in B-cells 1	NM_001165412, NM_003998
F07	NFKBIA	nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, alpha	NM_020529
G07	NOTCH1	notch 1	NM_017617
H07	NTRK1	neurotrophic tyrosine kinase, receptor, type 1	NM_001007792, NM_001012331, NM_002529
A08	NTRK3	neurotrophic tyrosine kinase, receptor, type 3	NM_001007156, NM_001012338, NM_001243101, NM_002530
B08	PDGFRA	platelet-derived growth factor receptor, alpha polypeptide	NM_006206
C08	PDGFRB	platelet-derived growth factor receptor, beta polypeptide	NM_002609
D08	PLAU	plasminogen activator, urokinase	NM_001145031, NM_002658
E08	PLAUR	plasminogen activator, urokinase receptor	NM_001005376, NM_001005377, NM_001301037, NM_002659
F08	PTEN	phosphatase and tensin homolog	NM_000314
G08	RAF1	Raf-1 proto-oncogene, serine/threonine kinase	NM_002880
H08	RARA	retinoic acid receptor, alpha	NM_000964, NM_001024809, NM_001145301, NM_001145302
A09	RB1	retinoblastoma 1	NM_000321
B09	RET	ret proto-oncogene	NM_020630, NM_020975

AccuPower® qPCR Array System: Human Cancer qPCR Panel kit

Well	Gene symbol	Description	Accession #
C09	RUNX1	runt-related transcription factor 1	NM_001001890, NM_001122607, NM_0011754
D09	SERPINB5	serpin peptidase inhibitor, clade B (ovalbumin), member 5	NM_002639
E09	SERPINE1	serpin peptidase inhibitor, clade E (nexin, plasminogen activator inhibitor type 1), member 1	NM_000602
F09	SYK	spleen tyrosine kinase	NM_001135052, NM_001174167, NM_001174168, NM_003177
G09	TAL1	T-cell acute lymphocytic leukemia 1	NM_001287347, NM_001290403, NM_001290404, NM_001290405, NM_001290406, NM_003189
H09	TERT	telomerase reverse transcriptase	NM_001193376, NM_198253
A10	TFE3	transcription factor binding to IGHM enhancer 3	NM_001282142, NM_006521
B10	TFEB	transcription factor EB	NM_001167827, NM_001271943, NM_001271944, NM_001271945, NM_007162
C10	TGFB1	transforming growth factor, beta 1	NM_000660
D10	TGFR1	transforming growth factor, beta receptor 1	NM_001130916, NM_004612
E10	THBS1	thrombospondin 1	NM_003246
F10	TIMP1	TIMP metalloproteinase inhibitor 1	NM_003254
G10	TIMP2	TIMP metalloproteinase inhibitor 2	NM_003255
H10	TLX1	T-cell leukemia homeobox 1	NM_001195517, NM_005521
A11	TNF	tumor necrosis factor	NM_000594
B11	TNFRSF10B	tumor necrosis factor receptor superfamily, member 10b	NM_003842, NM_147187
C11	TNFRSF1A	tumor necrosis factor receptor superfamily, member 1A	NM_001065
D11	TNFRSF25	tumor necrosis factor receptor superfamily, member 25	NM_001039664, NM_003790, NM_148965, NM_148966, NM_148967, NM_148970
E11	TP53	tumor protein p53	NM_000546, NM_001126112, NM_001126113, NM_001126114, NM_001126115, NM_001126116, NM_001126117, NM_001126118, NM_001276695, NM_001276696, NM_001276697, NM_001276698, NM_001276699, NM_001276760, NM_001276761
F11	TRADD	TNFRSF1A-associated via death domain	NM_003789
G11	VEGFA	vascular endothelial growth factor A	NM_001025366, NM_001025367, NM_001025368, NM_001025369, NM_001025370, NM_001033756, NM_001171622, NM_001171623, NM_001171624, NM_001171625, NM_001171626, NM_001171627, NM_001171628, NM_001171629, NM_001171630, NM_001204384, NM_001204385, NM_001287044, NM_003376
H11	WT1	Wilms tumor 1	NM_000378, NM_001198551, NM_001198552, NM_024424, NM_024426

Technical Support

- Address: 8-11, Munpyeongseoro, Daedeok-gu, Daejeon 34302, Republic of Korea Bioneer Gene Expression Analysis Team
- E-mail: qPCRarray@bioneer.com
- Phone: +82-42-930-8673 (GMT+9 09:00 to 18:00; Monday to Friday).

Reference

[1] Bustin, S.A., et al. 2009. The MIQE Guidelines: Minimum Information for Publication of Quantitative Real-Time PCR Experiments. *Clinical Chemistry* 55:4, 611-622

AccuPower® qPCR Array System: Immune qPCR Panel kit

Description

Our Immune qPCR panel kit can be used to analyze various genes related to immune pathways at once.

The primers in these panels have been meticulously tested to minimize non-specific reactions and show high sensitivity and reproducibility for a target gene.

Easily analyze various types of genes related to immune pathways by mixing the cDNA samples, and our *AccuPower® 2X GreenStar™* Master Mix in the panels coated with our validated primer sets.

Diseases related to immunology can be divided into several types: hyperimmune reactions such as allergy and asthma, immunodeficiency reactions such as AIDS, and autoimmune reactions such as rheumatic diseases and diabetes. When people age, gradual changes in immune homeostasis may occur, affecting the antigen recognition and the immune response, which may also lead to various dysfunctions in immune systems such as immune rejection responses during organ transplantation. Genes for the panels are all related to immunity and selected from the following categories related to anticancer immunity, antiviral activity, and immune senescence.

Features and Benefits

- 1. Accurate**
Shows high specificity and efficacy by using the panels with the primers meeting the MIQE (Minimum Information for Publication of Quantitative Real-Time PCR Experiments) guidelines^[1] to show accurate qPCR data (Figure 1).
- 2. Fast & economical**
Save valuable time and money for making and synthesizing primers for analysis and verifying them.
- 3. Convenient**
Perform the qPCR analysis only by using the cDNA samples and *AccuPower® 2X GreenStar™* Master Mix.

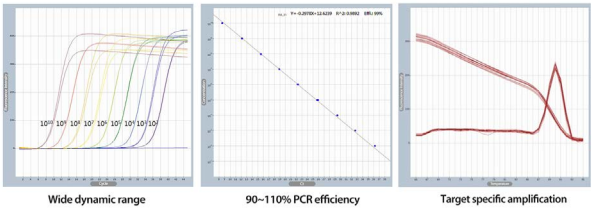


Figure 1. Validated primer data: wide dynamic range, one peak dissociation curve & PCR efficiency (Example. AKT gene).

Ordering information

Cat. No.	Product description
S-6042-PH2	AccuPower® qPCR Array System: Human Immune qPCR panel
S-6042-PM2	AccuPower® qPCR Array System: Mouse Immune qPCR panel

[Signaling pathways]

Table 1. Immune qPCR panel kit signaling pathways

Anticancer immunity	Immune cell	NK cell	CD3E, CD8A, FCGR3A, PTPRC, NCAM1, KLRK1, ITGA2, KLRD1
		DC cell	ITGAX, FCGR3A, HLA-DRA, CD1A, CCR7, CD80, CD86, CD83, CD209
		Cytotoxic T cell	CD8A, PDCD1, IL2RB, IL7R, LAG3, CD3E, CD5, CD7
		Macrophages	CD14, CD68, CD36, CD163, MRC1, CSF2RA, LAMP2, ITGAM, FCGR1A, ITGAL, ITGAX, CD69
	Cytokines, Chemokines and others	Cytokines and Chemokines	IL1A, IL1B, TNF, IL12B, IL6, IFNA1, IL2, IL4, TGFB1, GMCSF, IFNG, IL27, IL10, IFNAR2, CCL5, IL17A, IL7, CXCL8, CCL3, CCL4, GranzymeB
		Others	JUN, NFKB1, AKT1, HLA-A, HLA-B, HLA-C, TLR 3, TLR 7, TLR 8, TLR 9, CD40LG
Antiviral activity		MX1, OAS2, EIF2AK2, ADAR, ISG15, ISG20, IRF3, IRF7, IFNB1, IFIH1	
Immuno senescence		CD80, CD86, FAS, CD4, CD8A, CD28, CCR2, CCR7, CXCR4	
ETC		CD19, CXCR3, FoxP3, CCR4, CCR5, CTLA4, MKI67, IL2RA	

AccuPower® qPCR Array System: Immune qPCR Panel kit

Plate Map

A. Human Plate Map

	1	2	3	4	5	6	7	8	9	10	11	12
A	CD3E	CD11c	PD-1	CD36	CD69	IRF7	IFNA1	IFNAR2	JUN (AP1)	TLR8	CD19	ACTB
B	CD8A	HLA-DRA	IL2RB	CD163	MX1	IFNB1	IL2	CCL5	NFKB1	TLR9	CXCR3	B2M
C	CD16A	CD1A	IL7R	CD206	OAS2	MDA5	IL4	IL17A	AKT	CD40LG	FoxP3	GAPDH
D	CD45	CCR7	LAG3	CD116	PKR	IL1A	TGFB1	IL7	HLA-A	CD95	CCR4	HPRT1
E	CD56	CD80	CD5	CD107b	ADAR	IL1B	GMCSF	IL8	HLA-B	CD4	CCR5	RPLP0
F	CD314	CD86	CD7	CD11b	ISG15	TNF-α	IFNG	CCL3	HLA-C	CD28	CTLA4	GDC
G	CD49b	CD83	CD14	CD64	ISG20	IL12B	IL27	CCL4	TLR3	CCR2	KI-67	NTC
H	CD94	CD209	CD68	CD11a	IRF3	IL6	IL10	GranzymeB	TLR7	CXCR4	CD25	PPC

B. Mouse Plate Map

	1	2	3	4	5	6	7	8	9	10	11	12
A	Cd3e	Itgax	Il2rb	Cd36	Cd69	Ifnb1	Il2	Ifnar2	Jun	Tlr8	Cd19	Actb
B	Cd8a	H2-Ea-ps	Il7r	Cd163	Mx1	Ifih1	Il4	Ccl5	Nfkb1	Tlr9	Cxcr3	B2m
C	Fcgr4	Cd1d1	Lag3	Mrc1	Oas2	Il1a	Il25	Il17a	Akt1	Cd40lg	Foxp3	Gapdh
D	Ptprc	Ccr7	Cd2	Csf2ra	Elf2ak2	Il1b	Tgfb1	Il7	H2-K1	Fas	Ccr4	Hprt
E	Ncam1	Cd80	Cd5	Lamp2	Isig15	Tnf	Csf2	Cxcl1	Hif1a	Cd4	Ccr5	Rpl13a
F	Klrl1	Cd83	Cd7	Itgam	Isig20	Il12a	Ifng	Ccl3	H2-D1	Cd28	Ctla4	GDC
G	Itga2	Cd209a	Cd14	Fcgr1	Irf3	Il12b	Il27	Ccl4	Tlr3	Ccr2	Il2ra	NTC
H	Klrd1	Pdcd1	Cd68	Itgal	Irf7	Ifna1	Il10	Gzmb	Tlr7	Cxcr4	Il6	PPC





- Reference genes (internal control)**
 **Ref.** We offer 5 representative genes used as internal controls for relative quantitative analysis.
- Genomic DNA Control (GDC)**
 **GDC.** We provide genomic DNA control to determine whether a sample is gDNA contaminated.
- No Template Control (NTC)**
 **NTC.** We provide no template control to check for contamination during the experiment.
- Positive PCR Control**
 **PPC.** Positive PCR control checks the amplification efficiency of qPCR with the prepared template and primer.

Figure 2. Layout of Human Immune qPCR Panel

Human Gene list

Well	Gene symbol	Description	Accession #
A01	CD3E	CD3e molecule	NM_000733
B01	CD8A	CD8a molecule	NM_001145873, NM_001768, NM_171827
C01	FCGR3A	Fc fragment of IgG receptor IIIa	NM_000569, NM_001127592, NM_001127593, NM_001127595, NM_001127596, NM_001127596, NM_001127595, NM_001127593, NM_001127592, NM_000569
D01	PTPRC	protein tyrosine phosphatase receptor type C	NM_002838
E01	NCAM1	neural cell adhesion molecule 1	NM_001242608, NM_001242607, NM_001076682, NM_181351, NM_000615
F01	KLRK1	killer cell lectin like receptor K1	NM_007360, NM_001199805
G01	ITGA2	integrin subunit alpha 2	NM_002203
H01	KLRD1	killer cell lectin like receptor D1	NM_001114396, NM_002262
A02	ITGAX	integrin subunit alpha X	NM_000887
B02	HLA-DRA	major histocompatibility complex, class II, DR alpha	NM_019111

AccuPower® qPCR Array System: Immune qPCR Panel kit

Well	Gene symbol	Description	Accession #
C02	CD1A	CD1a molecule	NM_001763
D02	CCR7	C-C motif chemokine receptor 7	NM_001838
E02	CD80	CD80 molecule	NM_005191
F02	CD86	CD86 molecule	NM_001206925, NM_176892, NM_175862, NM_006889.4
G02	CD83	CD83 molecule	NM_001251901, NM_001040280, NM_004233
H02	CD209	CD209 molecule	NM_001144899, NM_001144897, NM_001144895, NM_021155.3
A03	PDCD1	programmed cell death 1	NM_005018.2
B03	IL2RB	interleukin 2 receptor subunit beta	NM_000878, NM_001172226, NM_001172225
C03	IL7R	interleukin 7 receptor	NM_002185
D03	LAG3	lymphocyte activating 3	NM_002286
E03	CD5	CD5 molecule	NM_014207
F03	CD7	CD7 molecule	NM_006137
G03	CD14	CD14 molecule	NM_001174105, NM_001174104, NM_001040021, NM_000591.3
H03	CD68	CD68 molecule	NM_001040059, NM_001251
A04	CD36	CD36 molecule	NM_001289909, NM_001289908, NM_001127444, NM_001127443, NM_000072, NM_001001547, NM_001001548
B04	CD163	CD163 molecule	NM_203416
C04	MRC1	mannose receptor C-type 1	NM_002438
D04	CSF2RA	colony stimulating factor 2 receptor alpha subunit	NM_001161531, NM_001161530, NM_001161529, NM_172249, NM_172247, NM_172246, NM_172245, NM_006140
E04	LAMP2	lysosomal associated membrane protein 2	NM_002294
F04	ITGAM	integrin subunit alpha M	NM_001145808, NM_000632
G04	FCGR1A	Fc fragment of IgG receptor Ia	NM_000566, NM_001301128, NM_012301
H04	ITGAL	integrin subunit alpha L	NM_002209
A05	CD69	CD69 molecule	NM_001781
B05	MX1	MX dynamin like GTPase 1	NM_001282920, NM_001178046, NM_002462, NM_001144925
C05	OAS2	2'-5'-oligoadenylate synthetase 2	NM_002535, NM_016817
D05	EIF2AK2	eukaryotic translation initiation factor 2 alpha kinase 2	NM_001135652, NM_002759, NM_001135651
E05	ADAR	eukaryotic translation initiation factor 2 alpha kinase 2	NM_001025107, NM_001193495, NM_015841, NM_015840, NM_001111
F05	ISG15	ISG15 ubiquitin like modifier	NM_005101
G05	ISG20	interferon stimulated exonuclease gene 20	NM_002201
H05	IRF3	interferon regulatory factor 3	NM_001197127, NM_001197125, NM_001197124, NM_001197123, NM_001197122, NM_001571
A06	IRF7	interferon regulatory factor 7	NM_001572, NM_004031, NM_004029
B06	IFNB1	interferon beta 1	NM_002176
C06	IFIH1	interferon induced with helicase C domain 1	NM_022168
D06	IL1A	interleukin 1 alpha	NM_000575
E06	IL1B	interleukin 1 beta	NM_000576
F06	TNF	tumor necrosis factor	NM_000594
G06	IL12B	interleukin 12B	NM_002187
H06	IL6	interleukin 6	NM_000600
A07	IFNA1	interferon alpha 1	NM_024013
B07	IL2	interleukin 2	NM_000586, NM_015057

AccuPower® qPCR Array System: Immune qPCR Panel kit

Well	Gene symbol	Description	Accession #
C07	IL4	interleukin 4	NM_172348, NM_000589, NM_032340
D07	TGFB1	transforming growth factor beta 1	NM_000660
E07	CSF2	colony stimulating factor 2	NM_000758
F07	IFNG	interferon gamma	NM_000619
G07	IL27	interleukin 27	NM_145659
H07	IL10	interleukin 10	NM_000572
A08	IFNAR2	interferon alpha and beta receptor subunit 2	NM_001289128, NM_001289126, NM_001289125, NM_207585
B08	CCL5	C-C motif chemokine ligand 5	NM_001278736, NM_002985
C08	IL17A	interleukin 17A	NM_002190
D08	IL7	interleukin 7	NM_000880, NM_001199886, NM_001199887, NM_001199888.1
E08	CXCL8	C-X-C motif chemokine ligand 8	NM_000584
F08	CCL3	C-C motif chemokine ligand 3	NM_002983
G08	CCL4	C-C motif chemokine ligand 4	NM_207007, NM_002984, NM_001291475, NM_001291473, NM_001291472, NM_001291471, NM_001291470, NM_001291469, NM_001291468
H08	GZMB	granzyme B	NM_004131
A09	JUN	Jun proto-oncogene, AP-1 transcription factor subunit	NM_002228
B09	NFKB1	nuclear factor kappa B subunit 1	NM_001165412, NM_003998
C09	AKT1	AKT serine/threonine kinase 1	NM_001014432, NM_001014431, NM_005163
D09	HLA-A	major histocompatibility complex, class I, A	NM_002116, NM_001242758, NM_012471
E09	HLA-B	major histocompatibility complex, class I, B	NM_005514, NM_002116, NM_001242758, NM_002127
F09	HLA-C	major histocompatibility complex, class I, C	
G09	TLR3	toll like receptor 3	NM_003265
H09	TLR7	toll-like receptor 7	NM_016562
A10	TLR8	toll-like receptor 8	NM_016610, NM_138636
B10	TLR9	toll-like receptor 9	NM_017442
C10	CD40LG	CD40 ligand	NM_000074
D10	FAS	Fas cell surface death receptor	NM_152872, NM_152871, NM_000043
E10	CD4	CD4 molecule	NM_001195017, NM_001195016, NM_001195015, NM_000616, NM_001195014
F10	CD28	CD28 molecule	NM_001243078, NM_001243077, NM_006139
G10	CCR2	C-C motif chemokine receptor 2	NM_001123041
H10	CXCR4	C-X-C motif chemokine receptor 4	NM_003467, NM_001008540
A11	CD19	CD19 molecule	NM_001178098, NM_001770
B11	CXCR3	C-X-C motif chemokine receptor 3	NM_001142797, NM_001504
C11	FoxP3	forkhead box P3	NM_001114377, NM_014009
D11	CCR4	C-C motif chemokine receptor 4	NM_005508
E11	CCR5	C-C motif chemokine receptor 5	NM_000579, NM_001100168
F11	CTLA4	cytotoxic T-lymphocyte associated protein 4	NM_001037631, NM_005214
G11	MKI67	marker of proliferation Ki-67	NM_002417
H11	IL2RA	interleukin 2 receptor subunit alpha	NM_000417

AccuPower® qPCR Array System: Immune qPCR Panel kit

■ Mouse Gene list

Well	Gene symbol	Description	Accession #
A01	Cd3e	CD3 antigen, epsilon polypeptide	NM_007648
B01	Cd8a	CD8 antigen, alpha chain	NM_001081110, NM_009857
C01	Fcgr4	Fc receptor, IgG, low affinity IV	NM_144559
D01	Ptpnc	protein tyrosine phosphatase, receptor type, C	NM_001111316, NM_001268286, NM_011210
E01	Ncam1	neural cell adhesion molecule 1	NM_001081445, NM_001113204, NM_001311065, NM_010875
F01	Klrk1	killer cell lectin-like receptor subfamily K, member 1	NM_001083322, NM_001286018, NM_033078
G01	Itga2	integrin alpha 2	NM_008396
H01	Klrk1	killer cell lectin-like receptor, subfamily D, member 1	NM_010654
A02	Itgax	integrin alpha X	NM_001363984, NM_001363985, NM_021334
B02	H2-Ea-ps	histocompatibility 2, class II antigen E alpha, pseudogene	NM_010381
C02	Cd1d1	CD1d1 antigen	NM_007639
D02	Ccr7	chemokine (C-C motif) receptor 7	NM_001301713, NM_007719
E02	Cd80	CD80 antigen	NM_001359898, NM_009855
F02	Cd83	CD83 antigen	NM_001289915, NM_009856
G02	Cd209a	CD209a antigen	NM_133238
H02	Pdcd1	programmed cell death 1	NM_008798
A03	Il2rb	interleukin 2 receptor, beta chain	NM_008368
B03	Il7r	interleukin 7 receptor	NM_001355680, NM_008372
C03	Lag3	lymphocyte-activation gene 3	NM_008479
D03	Cd2	CD2 antigen	NM_013486
E03	Cd5	CD5 antigen	NM_007650
F03	Cd7	CD7 antigen	NM_009854
G03	Cd14	CD14 antigen	NM_009841
H03	Cd68	CD68 antigen	NM_001291058
A04	Cd36	CD36 molecule	NM_001159555, NM_001159556, NM_001159557, NM_001159558, NM_007643
B04	Cd163	CD163 antigen	NM_001170395, NM_053094
C04	Mrc1	mannose receptor, C type 1	NM_008625
D04	Csf2ra	colony stimulating factor 2 receptor, alpha, low-affinity (granulocyte-macrophage)	NM_009970
E04	Lamp2	lysosomal-associated membrane protein 2	NM_001017959, NM_001290485, NM_010685
F04	Itgam	integrin alpha M	NM_001082960, NM_008401
G04	Fcgr1	Fc receptor, IgG, high affinity I	NM_010186
H04	Itgal	integrin alpha L	NM_001253872, NM_001253873, NM_001253874, NM_008400
A05	Cd69	CD69 antigen	NM_001033122
B05	Mx1	MX dynamin-like GTPase 1	NM_010846
C05	Oas2	2'-5' oligoadenylate synthetase 2	NM_001347448, NM_145227
D05	Eif2ak2	eukaryotic translation initiation factor 2-alpha kinase 2	NM_011163
E05	Isg15	ISG15 ubiquitin-like modifier	NM_015783
F05	Isg20	interferon-stimulated protein	NM_001113527, NM_001291220, NM_001291221, NM_020583
G05	Irf3	interferon regulatory factor 3	NM_016849

AccuPower® qPCR Array System: Immune qPCR Panel kit

Well	Gene symbol	Description	Accession #
H05	Irf7	interferon regulatory factor 7	NM_001252600, NM_001252601, NM_016850
A06	Ifnb1	interferon beta 1, fibroblast	NM_010510
B06	Ifih1	interferon induced with helicase C domain 1	NM_001164477, NM_027835
C06	Il1a	interleukin 1 alpha	NM_010554
D06	Il1b	interleukin 1 beta	NM_008361
E06	Tnf	tumor necrosis factor	NM_001278601, NM_013693
F06	Il12a	interleukin 12a	NM_001159424, NM_008351
G06	Il12b	interleukin 12b	NM_001303244
H06	Ifna1	interferon alpha 1	NM_010502
A07	Il2	interleukin 2	NM_008366
B07	Il4	interleukin 4	NM_021283
C07	Il25	interleukin 25	NM_080729
D07	Tgfb1	transforming growth factor, beta 1	NM_011577
E07	Csf2	colony stimulating factor 2 (granulocyte-macrophage)	NM_009969
F07	Ifng	interferon gamma	NM_008337
G07	Il27	interleukin 27	NM_145636
H07	Il10	interleukin 10 [Mus musculus (house mouse)]	NM_010548
A08	Ifnar2	interferon (alpha and beta) receptor 2	NM_001110498, NM_001347258, NM_010509
B08	Ccl5	chemokine (C-C motif) ligand 5	NM_013653
C08	Il17a	interleukin 17A	NM_010552
D08	Il7	interleukin 7	NM_001313888, NM_001313889, NM_001313890, NM_008371
E08	Cxcl1	chemokine (C-X-C motif) ligand 1	NM_008176
F08	Ccl3	chemokine (C-C motif) ligand 3	NM_011337
G08	Ccl4	chemokine (C-C motif) ligand 4	NM_013652
H08	Gzmb	granzyme B	NM_013542
A09	Jun	jun proto-oncogene	NM_010591
B09	Nfkb1	nuclear factor of kappa light polypeptide gene enhancer in B cells 1, p105	NM_008689
C09	Akt1	thymoma viral proto-oncogene 1	NM_001165894, NM_001331107, NM_009652
D09	H2-K1	histocompatibility 2, D region locus 1	NM_010380
E09	Hif1a	hypoxia inducible factor 1, alpha subunit	NM_001313919, NM_001313920, NM_010431
F09	H2-D1	histocompatibility 2, K1, K region	NM_001001892, NM_001347346
G09	Tlr3	toll-like receptor 3	NM_001357316, NM_001357317, NM_126166
H09	Tlr7	toll-like receptor 7	NM_001290755, NM_001290756, NM_001290757, NM_001290758, NM_133211
A10	Tlr8	toll-like receptor 8	NM_001313760, NM_001313761, NM_133212
B10	Tlr9	toll-like receptor 9	NM_031178
C10	Cd40lg	CD40 ligand	NM_011616
D10	Fas	Fas (TNF receptor superfamily member 6)	NM_001146708, NM_007987
E10	Cd4	CD4 antigen	NM_013488
F10	Cd28	CD28 antigen	NM_007642
G10	Ccr2	chemokine (C-C motif) receptor 2	NM_009915
H10	Cxcr4	chemokine (C-X-C motif) receptor 4	NM_001356509, NM_009911

AccuPower® qPCR Array System: Immune qPCR Panel kit

Well	Gene symbol	Description	Accession #
A11	Cd19	CD19 antigen	NM_001357091, NM_009844
B11	Cxcr3	chemokine (C-X-C motif) receptor 3	NM_009910
C11	Foxp3	forkhead box P3	NM_001199347, NM_001199348, NM_054039
D11	Ccr4	chemokine (C-C motif) receptor 4	NM_009916
E11	Ccr5	chemokine (C-C motif) receptor 5	NM_009917
F11	Ctla4	cytotoxic T-lymphocyte-associated protein 4	NM_001281976, NM_009843
G11	Il2ra	interleukin 2 receptor, alpha chain	NM_008367
H11	Il6	interleukin 6	NM_001314054, NM_031168

Technical Support

- Address: 8-11, Munpyeongseoro, Daedeok-gu, Daejeon 34302, Republic of Korea Bioneer Gene Expression Analysis Team
- E-mail: qPCRarray@bioneer.com (GMT+9 09:00 to 18:00; Monday to Friday).

Reference

[1] Bustin, S.A., et al. 2009. The MIQE Guidelines: Minimum Information for Publication of Quantitative Real-Time PCR Experiments. *Clinical Chemistry* 55:4, 611-622

AccuPower® qPCR Array System: Immune checkpoint qPCR Panel

Description

Immune checkpoint qPCR panel can be used to analyze various immune checkpoint related genes at once. The primers in this product are designed to show high sensitivity and reproducibility for the target genes and tested with extensive quality tests to minimize non-specific reactions. Those validated primers are coated on the panel so that analysis for immune pathway genes can be easily initiated simply by adding cDNA samples and AccuPower® 2X GreenStar™ Master Mix.

This product also includes antigens for cancers with many different characteristics from its genetic and epigenetic changes. The immune system uses a wide variety of antigens to distinguish between "self" and "non-self." The quality of T cell response begins with antigen recognition by the T cell receptor (TCR), which is regulated by the co-stimulatory and inhibitory signal gene (Immune checkpoint gene). The genes in this panel are selected from immune checkpoint genes expressed from cancer cells, antigen-presenting cells (APC), and immune cells.

Features and Benefits

- 1. Accurate**
Shows high specificity and efficacy by using the panels with the primers meeting the MIQE (Minimum Information for Publication of Quantitative Real-Time PCR Experiments) guidelines^[1] to show accurate qPCR data (Figure 1).
- 2. Fast & economical**
Save valuable time and money for making and synthesizing primers for analysis and verifying them.
- 3. Convenient**
Perform the qPCR analysis only by using the cDNA samples and AccuPower® 2X GreenStar™ Master Mix.

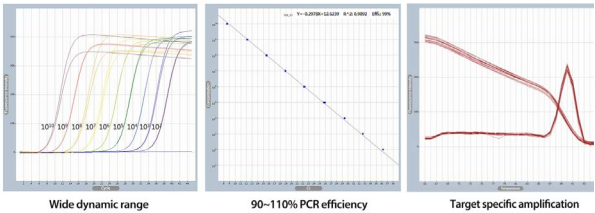


Figure 1. Validated primer data: wide dynamic range, one peak dissociation curve & PCR efficiency (Example. AKT gene).

Ordering Information

Cat. No.	Product Description
S-6042-PH3	AccuPower® qPCR Array System: Human Immune Checkpoint qPCR Panel

[Signaling pathways]

Table 1. Immune checkpoint qPCR panel kit signaling pathways

Immune cell	PDCD1, CD28, CTLA4, CD274, BTLA, TNFSF14, CD160, TNFRSF4, CD27, CD40LG, CSF1R, BTLA, TNFRSF9, ICOS, TNFRSF18, TIGIT, CD226, HAVCR2, CEACAM1, LAG3
Antigen presenting cell / Cancer cell	CD274, PDCD1LG2, CD80, CD86, TNFRSF14, TNFSF4, CD276, CD70, CD40, CSF1, IDO1, VTCN1, TNFSF9, ICOSLG, TNFSF18, PVR, LGALS1, LGALS3, LGALS9, CD47, CEACAM1

AccuPower® qPCR Array System: Immune checkpoint qPCR Panel

■ Plate Map

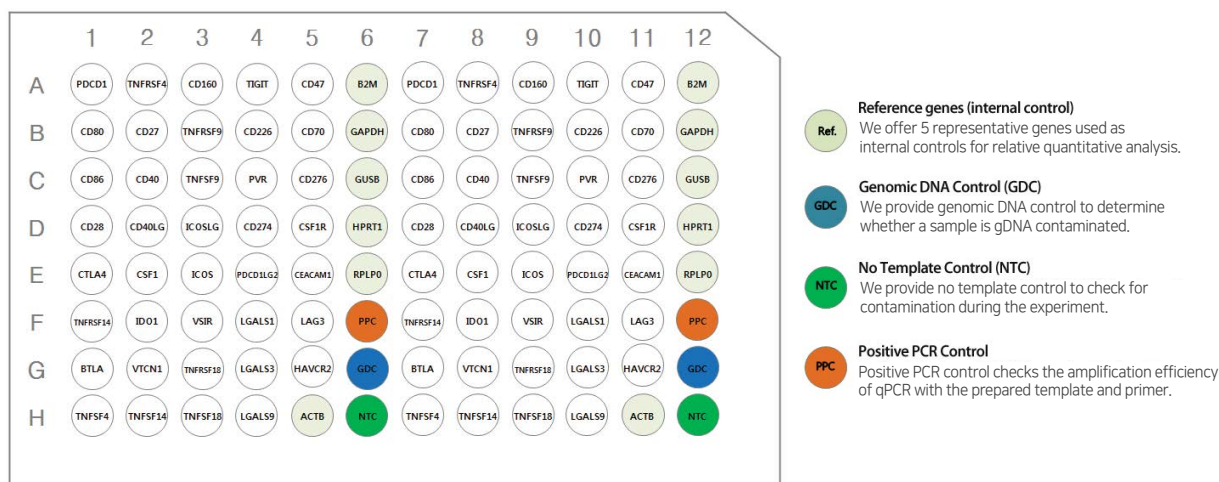


Figure 2. Layout of Immune checkpoint qPCR Panel.

■ Gene list

Well	Gene symbol	Description	Accession #
A01	PDCD1	Programmed cell death 1	NM_005018
B01	CD80	CD80 molecule	NM_005191
C01	CD86	CD86 molecule	NM_001206924, NM_001206925, NM_006889, NM_175862, NM_176892
D01	CD28	CD28 molecule	NM_001243077, NM_001243078, NM_006139.3
E01	CTLA4	Cytotoxic T-lymphocyte-associated protein 4	NM_001037631, NM_005214
F01	TNFRSF14	Tumor necrosis factor receptor superfamily, member 14	NM_001297605, NM_003820
G01	BTLA	B and T lymphocyte associated	NM_001085357, NM_181780
H01	TNFSF4	Tumor necrosis factor (ligand) superfamily, member 4	NM_001297562, NM_003326
A02	TNFRSF4	Tumor necrosis factor receptor superfamily, member 4	NM_003327
B02	CD27	CD27 molecule	NM_001242
C02	CD40	CD40 molecule, TNF receptor superfamily member 5	NM_001250, NM_001302753, NM_152854
D02	CD40LG	CD40 ligand	NM_000074
E02	CSF1	Colony stimulating factor 1	NM_000757, NM_172212, NM_172210, NM_172211
F02	IDO1	Indoleamine 2,3-dioxygenase 1	NM_002164
G02	VTCN1	V-set domain containing T cell activation inhibitor 1	NM_001253849, NM_001253850, NM_024626
H02	TNFSF14	Tumor necrosis factor superfamily member 14	NM_003807, NM_172014
A03	CD160	CD160 molecule	NM_007053
B03	TNFRSF9	Tumor necrosis factor receptor superfamily member 9	NM_001561
C03	TNFSF9	Tumor necrosis factor superfamily member 9	NM_003811
D03	ICOSLG	Inducible T-cell co-stimulator ligand	NM_001283050, NM_001283051, NM_001283052, NM_015259
E03	ICOS	Inducible T-cell co-stimulator	NM_012092
F03	VISR	V-set immunoregulatory receptor	NM_022153
G03	TNFRSF18	Tumor necrosis factor receptor superfamily member 18	NM_004195, NM_148901, NM_148902
H03	TNFSF18	Tumor necrosis factor superfamily member 18	NM_005092

AccuPower® qPCR Array System: Immune checkpoint qPCR Panel

Well	Gene symbol	Description	Accession #
A04	TIGIT	T-cell immunoreceptor with Ig and ITIM domains	NM_173799
B04	CD226	CD226 molecule	NM_001303618, NM_001303619, NM_006566
C04	PVR	Poliovirus receptor	NM_001135768, NM_001135769, NM_001135770, NM_006505
D04	CD274	CD274 molecule	NM_001267706, NM_014143
E04	PDCD1LG2	Programmed cell death 1 ligand 2	NM_025239
F04	LGALS1	Lectin, galactoside-binding, soluble, 1	NM_002305
G04	LGALS3	Lectin, galactoside-binding, soluble, 3	NM_001177388, NM_002306
H04	LGALS9	Lectin, galactoside-binding, soluble, 9	NM_002308, NM_009587
A05	CD47	CD47 molecule	NM_001777, NM_198793
B05	CD70	CD70 molecule	NM_001252
C05	CD276	CD276 molecule	NM_001024736, NM_025240
D05	CSF1R	Colony stimulating factor 1 receptor	NM_001288705, NM_005211
E05	CEACAM1	Carcinoembryonic antigen-related cell adhesion molecule 1 (biliary glycoprotein)	NM_001024912, NM_001184813, NM_001184815, NM_001184816, NM_001205344, NM_001712
F05	LAG3	Lymphocyte-activation gene 3	NM_002286
G05	HAVCR2	Hepatitis A virus cellular receptor 2	NM_032782

Technical Support

- Address: 8-11, Munpyeongseoro, Daedeok-gu, Daejeon 34302, Republic of Korea Bioneer Gene Expression Analysis Team
- E-mail: qPCRarray@bioneer.com (GMT+9 09:00 to 18:00; Monday to Friday).

Reference

[1] Bustin, S.A., et al. 2009. The MIQE Guidelines: Minimum Information for Publication of Quantitative Real-Time PCR Experiments. *Clinical Chemistry* 55:4, 611-622

AccuPower® qPCR Array System: Reference qPCR primer set

○ Description

AccuPower® qPCR Array System: Reference qPCR primer set contains primers of 16 types of human and mouse housekeeping genes mainly used for qPCR relative quantitative analysis. Reference genes, also called as housekeeping genes, are required for the maintenance of basic cellular functions. They are closely related to the survival of the cells and must be expressed consistently throughout all cells and living tissues of an organism. However, depending on the experimental conditions and sample types, their expression may be affected. Therefore, to attain accurate qPCR results, reference genes must be selected carefully depending on the circumstances of a research. Our Reference Primer Sets can be used to analyze the changes of expression levels depending on the various experimental conditions with ease. Those are currently being sold in eight different sets, further divided with set A and set B, for each species. Validated primer sets are also available for purchase in 8-tube strips, enough for 20 trials. (Figure 2).

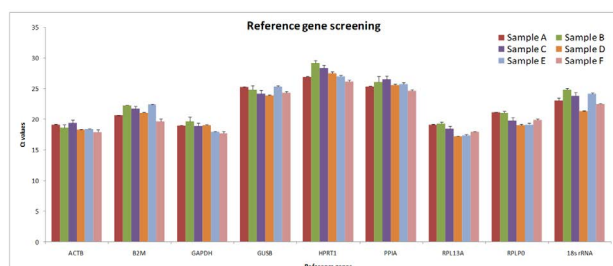


Figure 1. Reference gene screening

○ Features and Benefits

■ Convenient

Screen using 16 kinds of commonly used reference genes (A set: 8 genes / B set: 8 genes)

■ Fidelity

Contain qPCR primers with qPCR efficiency of 90 - 110% ensuring detection limit of 100 copies.

■ Economic

Reduce time and money for primer design, synthesis and efficiency assays

○ Application

Quantitative Real-time PCR

○ Ordering Information

Cat. No	Product Description
S-6042-S200	AccuPower® qPCR Array System: Single gene qPCR primer, 200 rxns/tube
S-6042-TH0A	AccuPower® qPCR Array System: Human Reference qPCR primer A set, 20 rxns
S-6042-TH0B	AccuPower® qPCR Array System: Human Reference qPCR primer B set, 20 rxns
S-6042-TM0A	AccuPower® qPCR Array System: Mouse Reference qPCR primer A set, 20 rxns
S-6042-TM0B	AccuPower® qPCR Array System: Mouse Reference qPCR primer B set, 20 rxns

○ Product Component

Reference qPCR primer A set and B set is composed of validated qPCR primers of different 16 housekeeping genes.

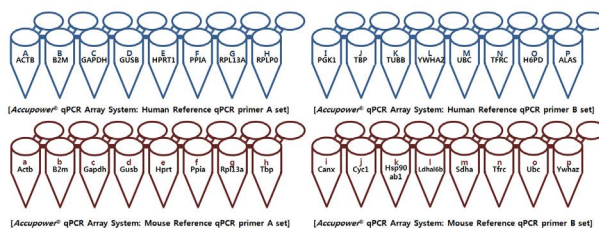


Figure 2. Product component of AccuPower® qPCR Array System: Reference qPCR primer set

○ Experimental Data

[Target specificity test]

Bioneer

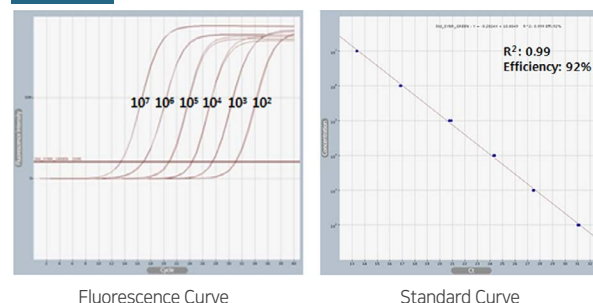


Figure 3. Target Specificity test using [BIONEER] Mouse template (Example. ACTB gene). It can be seen that specificity for the target gene and qPCR efficiency are within the range recommended by the MIQE guideline (100%±10%). Limit of detection (LOD) is found at 100 copies or less.

Competitor Q

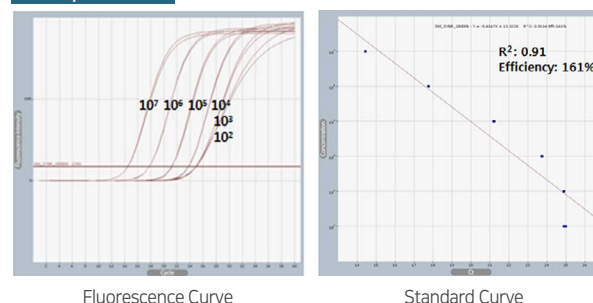


Figure 4. Target Specificity test using [Competitor] Mouse template (Example. ACTB gene). Aggregation is found in the efficiency fluorescence curve from 10⁴ copy. The efficiency is 161%, which is out of the qPCR efficiency range recommended by the MIQE guideline (100%±10%). This implies that its target specificity is lower than that of our company's.

AccuPower® qPCR Array System: Reference qPCR primer set

Gene List

Tube	Gene symbol	Description	Accession #
AccuPower® qPCR Array System: Human Reference qPCR primer A set			
A	ACTB	Actin, beta	NM_001101
B	B2M	Beta-2-microglobulin	NM_004048
C	GAPDH	Glyceraldehyde-3-phosphate dehydrogenase	NM_001256799, NM_001289745
D	GUSB	Glucuronidase, beta	NM_001289746, NM_002046
E	HPRT1	Hypoxanthine phosphoribosyltransferase 1	NM_000181, NM_001284290,
F	PPIA	Peptidylprolyl isomerase A (cyclophilin A)	NM_001293104, NM_001293105
G	RPL13A	Ribosomal protein L13a	NM_000194
H	RPLP0	Ribosomal protein, large, P0	NM_001300981, NM_021130
AccuPower® qPCR Array System: Human Reference qPCR primer B set			
I	ALAS1	5'-aminolevulinate synthase 1	NM_000688, NM_001304443, NM_001304444, NM_199166
J	G6PD	Glucose-6-phosphate dehydrogenase	NM_000402, NM_001042351
K	PGK1	Phosphoglycerate kinase 1	NM_000291
L	TBP	TATA box binding protein	NM_001172085, NM_003194
M	TFRC	Transferrin receptor	NM_001128148, NM_003234
N	TUBB	Tubulin, beta class I	NM_001293212, NM_001293213, NM_001293214, NM_001293215, NM_001293216, NM_178014
O	UBC	Ubiquitin C	NM_021009
P	YWHAZ	Tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, zeta	NM_001135699, NM_001135700, NM_001135701, NM_001135702, NM_003406, NM_145690
AccuPower® qPCR Array System: Mouse Reference qPCR primer A set			
a	Actb	Actin, beta	NM_007393
b	B2m	Beta-2 microglobulin	NM_009735
c	Gapdh	Glyceraldehyde-3-phosphate dehydrogenase	NM_001289726, NM_008084
d	Gusb	Glucuronidase, beta	NM_010368
e	Hprt	Hypoxanthine guanine phosphoribosyl transferase	NM_013556
f	Ppia	Peptidylprolyl isomerase A	NM_008907
g	Rpl13a	Ribosomal protein L13A	NM_009438
h	Tbp	TATA box binding protein	NM_013684
AccuPower® qPCR Array System: Mouse Reference qPCR primer B set			
i	Canx	Calnexin	NM_001110499, NM_001110500, NM_007597
j	Cyc1	Cytochrome c-1	NM_025567
k	Hsp90ab1	Heat shock protein 90 alpha (cytosolic), class B member 1	NM_008302
l	Ldhal6b	Lactate dehydrogenase A-like 6B	NM_175349
m	Sdha	Succinate dehydrogenase complex, subunit A, flavoprotein (Fp)	NM_023281
n	Tfrc	Transferrin receptor	NM_011638
o	Ubc	Ubiquitin C	NM_019639
p	Ywhaz	Tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, zeta	NM_001253805, NM_001253806, NM_001253807, NM_011740,

Technical Support

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Reference

- [1] Radoni A, Thulke S, Mackay IM, et al. 2004. Guideline to reference gene selection for quantitative real-time PCR. *Biochem Biophys Res Commun*. 23;313(4):856-62
- [2] Svingen T, Letting H, Hadrup N, et al. 2015. Selection of reference genes for quantitative RT-PCR (RT-qPCR) analysis of rat tissues under physiological and toxicological conditions. *PeerJ*. 24;3:e855

AccuPower® Human 5-plex Reference qPCR Primer and Probe Set

Description

Human 5-plex Reference qPCR primer and probe set contains 15 types of human and mouse reference gene (housekeeping gene) that are mainly used for qPCR relative quantity analysis. Reference genes, also called as housekeeping genes, are required for the maintenance of basic cellular functions. They are closely related to the survival of the cells and must be expressed consistently throughout all cells and living tissues of an organism. However, depending on the experimental conditions and sample types, their expression may be affected. Therefore, to attain accurate qPCR results, reference genes must be selected carefully depending on the circumstances of a research. Our Reference Primer Sets can be used to analyze the changes of expression levels depending on the various experimental conditions.

Our Human 5-plex Reference qPCR primer and probe set is a multiplex qPCR product that can be used to detect five types of human reference in one qPCR tube.

*This product is optimized for Exicycler™ 96 and AccuPower® Plus DualStar™.

Features and Benefits

1. Accurate

Shows high specificity and efficacy by using the panels with the primers meeting the MIQE (Minimum Information for Publication of Quantitative Real-Time PCR Experiments) guidelines^[1] to show accurate qPCR data (Figure 1).

2. fast & economical

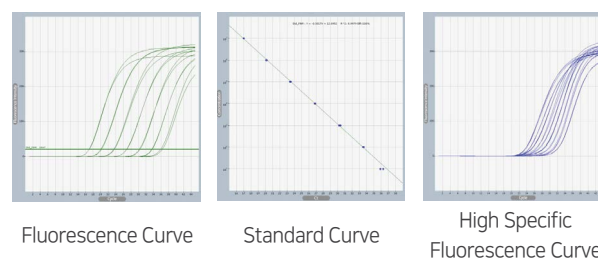
- Save time and money for primer, probe design and validation.
- Multiplex qPCR-optimized products can minimize the amounts of samples and time required for analysis.

Ordering Information

Cat. No	Product Description
S-6043-SH0	AccuPower® qPCR Array System: Human 5-plex Reference qPCR primer and probe set., 20 rxns

Experimental Data

1)



2)

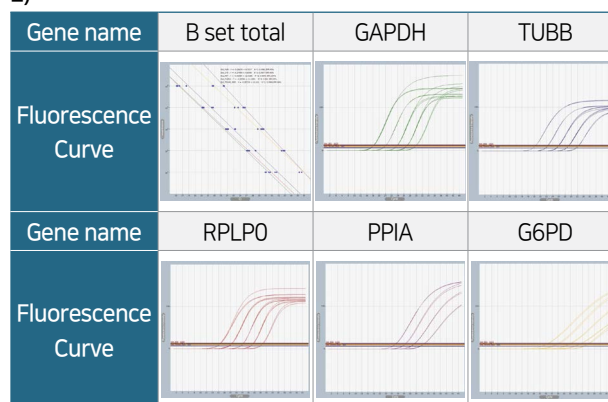


Figure 1. A) Result of validation of probe/qPCR primer through hydrolysis probe method. High amplification efficiency was shown in $10^7 - 10^1$ range. R^2 : 0.99, qPCR efficiency: 100% (Ex. TUBB gene)
B) Result from 5-plex qPCR with 500 ng of total RNA from HeLa cell. All genes with 10-fold dilution showed amplification efficiency of 100% ($\pm 10\%$) from cDNA.

AccuPower® Human 5-plex Reference qPCR Primer and Probe Set

■ Gene List

Set	Dye	Gene symbol	Description	Accession #
A set	FAM	ACTB	actin, beta	NM_001101
	TEXAS-RED	B2M	beta-2-microglobulin	NM_004048
	Cyanine 5	TFRC	transferrin receptor	NM_001128148, NM_003234
	TET	GUSB	glucuronidase, beta	NM_000181, NM_001284290, NM_001293104, NM_001293105
	TAMRA	HPRT1	hypoxanthine phosphoribosyltransferase 1	NM_000194
B set	FAM	GAPDH	glyceraldehyde-3-phosphate dehydrogenase	NM_001256799, NM_001289745, NM_001289746, NM_002046
	TEXAS-RED	TUBB	tubulin, beta class I	NM_001293212, NM_001293213, NM_001293214, NM_001293215, NM_001293216, NM_178014
	Cyanine 5	RPLP0	ribosomal protein, large, P0	NM_001002, NM_053275
	TET	PPIA	peptidylprolyl isomerase A (cyclophilin A)	NM_001300981, NM_021130
	TAMRA	G6PD	glucose-6-phosphate dehydrogenase	NM_000402, NM_001042351
C set	FAM	TBP	TATA box binding protein	NM_001172085, NM_003194
	TEXAS-RED	PGK1	phosphoglycerate kinase 1	NM_000291
	Cyanine 5	YWHAZ	tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, zeta	NM_001135699, NM_001135700, NM_001135701, NM_001135702, NM_003406, NM_145690
	TET	ALAS1	5'-aminolevulinate synthase 1	NM_000688, NM_001304443, NM_001304444, NM_199166
	TAMRA	RPL13A	ribosomal protein L13a	NM_001270491, NM_012423

○ Technical Support

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Reference

- [1] Radoni A, Thulke S, Mackay IM, *et al.* 2004. Guideline to reference gene selection for quantitative real-time PCR. *Biochem Biophys Res Commun.* 23;313(4):856-62
- [2] Svingen T, Letting H, Hadrup N, *et al.* 2015. Selection of reference genes for quantitative RT-PCR (RT-qPCR) analysis of rat tissues under physiological and toxicological conditions. *PeerJ.* 24;3:e855

Single Gene qPCR Primer Set

Description

Single Gene qPCR Primer Set is a service for checking the ds-DNA binding dye type primers according to MIQE (Minimum Information for Publication of Quantitative Real-Time PCR Experiments) guideline* whether it is suitable to be used for real-time PCR analysis. By providing us with the gene information that you would like to analyze, we verify the primer designs, target specificity, and PCR efficiency according to the guidelines written above, and then, provide 200 rxn of primer sets. Furthermore, we can also undergo this validation tests using the sequences of the primers that you have.

Therefore, the primers that are delivered from this service can be used in laboratories directly without any further validation tests to get ready-to-publish data.

*The MIQE Guidelines [Bustin, S.A., et al. 2009. The MIQE Guidelines: Minimum Information for Publication of Quantitative Real-Time PCR Experiments, Clinical Chemistry 55:4, 611-622]

Table. Information required for qPCR validation according to the MIQE guideline

Item to check
Specificity (gel, sequence, melt, or digest)

For dsDNA binding dyes, C _q of the NTC
Calibration curves with slope and y intercept
PCR efficiency calculated from slope
R ² of calibration curve
Linear dynamic range
C _q variation at LOD
Evidence for LOD

Features and Benefits

- Target-specific primer design using primer blast and our - bioinformatics tool
- Exclusion of self primer-dimer formation sequence
- Identification of single peak in the dissociation curve
- Identification of single band in Gel electrophoresis
- Short amplicon size of 70~150 bp
- Wide amplification range of copies being about 10² ~ 10⁷
- qPCR amplification efficiency of 90-110% in compliance with the MIQE Guideline

Application

Applicable to Quantitative Real-Time PCR

Experimental Data

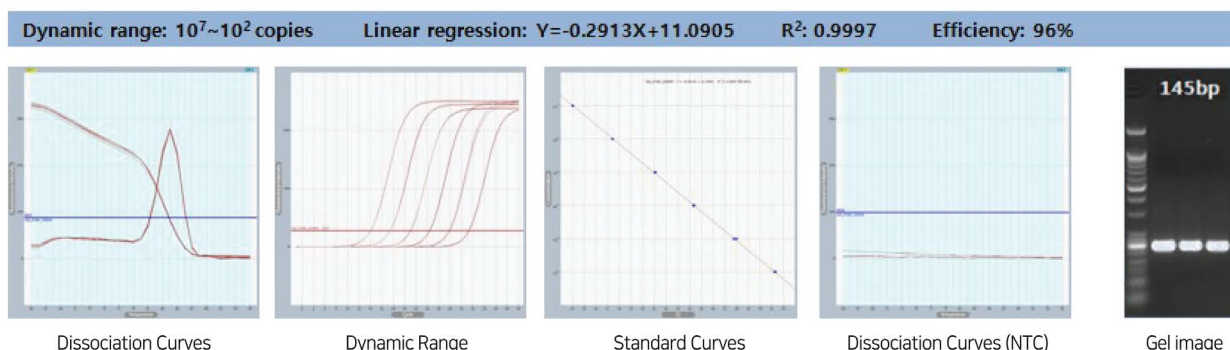


Figure 1. A diagram showing dissociation curve, dynamic range, standard curve, and electrophoresis image of the human ACTB and its target.

*As Single Gene qPCR Primer Set is verified with *Exicycler*™ 96 V3 and 2X *GreenStar*™ qPCR Master Mix (-ROX Dye)(K-6253), results may be different if other company products are used.

Ordering Information

Cat. No	Product Description
S-6042-S200	Single Gene qPCR Primer Set
Related Products	
K-3140	<i>AccuPrep</i> ® Universal RNA Extraction Kit
K-2201	<i>AccuPower</i> ® <i>RocketScript</i> ™ Cycle RT PreMix & Master Mix
K-6254	<i>AccuPower</i> ® 2X <i>GreenStar</i> ™ qPCR Master Mix (-ROX Dye) (5ml)

* Single Gene qPCR Primer Set is optimized for the above products.