USER GUIDE

AccuPower® qPCR Array System: Human Cancer qPCR panel kit (96 well plate)

Cat. No. S-6042-PH1

BIONEER
Innovation • Value • Discovery

AccuPower® qPCR Array System: Human Cancer qPCR panel kit (96 well plate)

User Guide

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Σ 1

Version No.: 2 (2022-06-02)

Please read all the information in booklet before using the unit

BIONEER Corporation

8-11, Munpyeongseo-ro, Daedeok-gu, Daejeon 34302, Republic of Korea

Tel: +82-42-930-8777

Fax: +82-42-930-8688

Email: sales@bioneer.co.kr

www.bioneer.com

Intended Use

AccuPower® qPCR Array System: Human Cancer qPCR panel kit is developed and supplied for research purposes only. Certain applications possible with this kit may require special approval by appropriate local and/or national regulatory authorities in the country of use.

Safety Warning and Precaution

Wear appropriate protection when handling any irritant or harmful reagents. The use of a laboratory coat, protective gloves and safety goggles are highly recommended. For more information, please consult the appropriate Material Safety Data Sheet (MSDS).

Warranty and Liability

All BIONEER products undergo extensive Quality Control testing and validation. BIONEER guarantees quality during the warranty period as specified, when following the appropriate protocol as supplied with the product. It is the responsibility of the purchaser to determine the suitability of the product for its particular use. Liability is conditional upon the customer providing full details of the problem to BIONEER within 30 days.

Quality Management System ISO 9001 Certified

Every aspect of our quality management system from product development, production to quality assurance and supplier qualification meets the world-class standards.

Trademark

AccuPower® is a registered trademark of BIONEER Corporation.

Copyright

Notice

BIONEER Corporation reserves the right to make corrections, modifications, improvements and other changes to its products, services, specifications or product descriptions at any time without notice.

Contents

Product Information

Components

Components	Amount
AccuPower® qPCR Array System: Human Cancer qPCR panel kit (96 well plate)	1 plate
Adhesive Optical Sealing Film	1 sheet per plate

Storage

This product is lyophilized and shipped at ambient temperature. Store at room temperature without direct sunlight for long term storage. If stored in the recommended temperature, this product will be stable for 2 years after the delivery date.

Plate Map

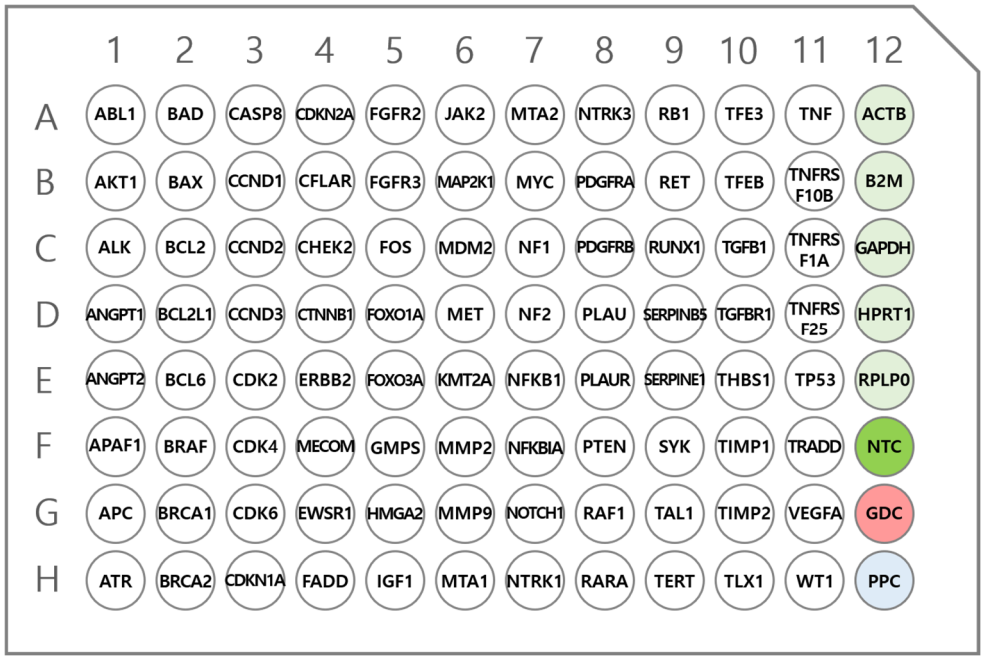

Figure 1. Layout of Human Cancer qPCR panel kit. The panel is involved 88 target genes (A1 through H11), 5 reference genes (A12-E12), and 3 control primers (F12-H12).

Control Primers

1. Non-Template Control (NTC)

- NTC is a negative control for checking on random or on reagent contamination.
- Just add the pre-mixture containing nuclease-free water but excluding the template into NTC well.
- If the value of Ct^{NTC} is less than 35, there is overall DNA contamination in your PCR system. In this case, clean up the equipment and replace all the reagents to new ones.

2. Genomic DNA Control (GDC)

- GDC primer is for the detection of non-transcribed genomic DNA contamination.
- In GDC well, primers which target genomic DNA are coated.
- Add pre-mixture (your template, 2X Master Mix, and nuclease free water) into the GDC well.
- If the value of Ct^{GDC} is less than 35, gDNA contamination might have occurred in your RNA samples. In this case, you ought to conduct an additional DNase treatment to clean up your samples.

3. Positive PCR Control (PPC)

- PPC primer is for the PCR test.
- The PPC well contains positive template and primers, so just add 2X Master Mix and nuclease-free water into the PPC well.
- The value of Ct^{PPC} should be referred to the quick manual provided together.

Gene Table

Well	Gene symbol	Accession #	Description	Also known as
A1	ABL1	NM_005157, NM_007313	ABL proto-oncogene 1, non-receptor tyrosine kinase	RP11-83J21.1, ABL, JTK7, bcr/abl, c-ABL, c-ABL1, p150, v-abl
B1	AKT1	NM_001014431, NM_001014432, NM_005163	v-akt murine thymoma viral oncogene homolog 1	AKT, CWS6, PKB, PKB-ALPHA, PRKBA, RAC, RAC-ALPHA
C1	ALK	NM_004304	Anaplastic lymphoma receptor tyrosine kinase	CD246, NBLST3
D1	ANGPT1	NM_001146, NM_001199859	Angiopoietin 1	AGP1, AGPT, ANG1
E1	ANGPT2	NM_001118887, NM_001118888, NM_001147	Angiopoietin 2	AGPT2, ANG2
F1	APAF1	NM_001160, NM_013229, NM_181861, NM_181868, NM_181869	Apoptotic peptidase activating factor 1	APAF-1, CED4
G1	APC	NM_000038, NM_001127510, NM_001127511	Adenomatous polyposis coli	AI047805, AU020952, AW124434, CC1, Min, mAPC
H1	ATR	NM_001184	ATR serine/threonine kinase	FCTCS, FRP1, MEC1, SCKL, SCKL1
A2	BAD	NM_004322, NM_032989	BCL2-associated agonist of cell death	BBC2, BCL2L8
B2	BAX	NM_001291428, NM_001291429, NM_001291430, NM_001291431, NM_004324, NM_138761, NM_138763, NM_138764	BCL2-associated X protein	BCL2L4
C2	BCL2	NM_000633, NM_000657	B-cell CLL/lymphoma 2	Bcl-2, PPP1R50
D2	BCL2L1	NM_001191, NM_138578	BCL2-like 1	RP5-857M17.3, BCL-XL/S, BCL2L, BCLX, BCLXL, BCLXS, Bcl-X, PPP1R52, bcl-xL, bcl-xS
E2	BCL6	NM_001130845, NM_001134738, NM_001706	B-cell CLL/lymphoma 6	BCL5A, LAZ3, ZBTB27, ZNF51, BCL6
F2	BRAF	NM_004333	B-Raf proto-oncogene, serine/threonine kinase	B-RAF11, NS7, RAFB1, BRAF
G2	BRCA1	NM_007294, NM_007297, NM_007298, NM_007299, NM_007300	Breast cancer 1, early onset	BRCAI, BRCC1, BROVCA1, FANCS, IRIS, PNCA4, PPP1R53, PSCP, RNF53

H2	BRCA2	NM_000059	Breast cancer 2, early onset	RP11-298P3.4, BRCC2, BROVCA2, FACD, FAD, FAD1, FANCD, FANCD1, GLM3, PNCA2, XRCC11
A3	CASP8	NM_001080124, NM_001080125, NM_001228, NM_033355, NM_033356, NM_033358	Caspase 8, apoptosis-related cysteine peptidase	ALPS2B, CAP4, Casp-8, FLICE, MACH, MCH5
B3	CCND1	NM_053056	Cyclin D1	BCL1, D11S287E, PRAD1, U21B31
C3	CCND2	NM_001759	Cyclin D2	KIAK0002, MPPH3
D3	CCND3	NM_001136017, NM_001136125, NM_001136126, NM_001287427, NM_001287434, NM_001760	Cyclin D3	RP5-973N23.3
E3	CDK2	NM_001290230, NM_001798, NM_052827	Cyclin-dependent kinase 2	CDKN2, p33(CDK2)
F3	CDK4	NM_000075	Cyclin-dependent kinase 4	CMM3, PSK-J3
G3	CDK6	NM_001145306, NM_001259	Cyclin-dependent kinase 6	MCPH12, PLSTIRE
H3	CDKN1A	NM_000389, NM_001220777, NM_001220778, NM_001291549, NM_078467	Cyclin-dependent kinase inhibitor 1A (p21, Cip1)	CAP20, CDKN1, CIP1, MDA-6, P21, SDI1, WAF1, p21CIP1
A4	CDKN2A	NM_000077, NM_001195132, NM_058195, NM_058197	Cyclin-dependent kinase inhibitor 2A	ARF, CDK4I, CDKN2, CMM2, INK4, INK4A, MLM, MTS-1, MTS1, P14, P14ARF, P16, P16-INK4A, P16INK4, P16INK4A, P19, P19ARF, TP16
B4	CFLAR	NM_001127183, NM_001127184, NM_001202515, NM_001202516, NM_001202517, NM_001202518, NM_001202519, NM_003879	CASP8 and FADD-like apoptosis regulator	CASH, CASP8AP1, CLARP, Casper, FLAME, FLAME-1, FLAME1, FLIP, I-FLICE, MRIT, c-FLIP, c-FLIPL, c-FLIPR, c-FLIPS
C4	CHEK2	NM_001005735, NM_001257387, NM_007194, NM_145862	Checkpoint kinase 2	RP11-436C9.1, CDS1, CHK2, HuCds1, LFS2, PP1425, RAD53, hCds1
D4	CTNNB1	NM_001098209, NM_001098210,	Catenin (cadherin-associated protein), beta 1, 88kDa	OK/SW-cl.35, CTNNB, MRD19, armadillo

		NM_001904		
E4	ERBB2	NM_001005862, NM_001289936, NM_001289937, NM_001289938, NM_004448	Erb-b2 receptor tyrosine kinase	CD340, HER-2, HER-2/neu, HER2, MLN 19, NEU, NGL, TKR1
F4	MECOM	NM_001105077, NM_001105078, NM_001163999, NM_001164000, NM_001205194, NM_004991, NM_005241	MDS1 and EVI1 complex locus	hCG_1640438, AML1-EVI-1, EVI1, MDS1, MDS1-EVI1, PRDM3
G4	EWSR1	NM_001163285, NM_001163286, NM_001163287, NM_005243, NM_013986	EWS RNA-binding protein 1	AC002059.7, EWS, bK984G1.4
H4	FADD	NM_003824	Fas (TNFRSF6)-associated via death domain	GIG3, MORT1
A5	FGFR2	NM_000141, NM_001144913, NM_001144914, NM_001144915, NM_001144916, NM_001144917, NM_001144918, NM_001144919, NM_022970, NM_023029	Fibroblast growth factor receptor 2	BBDS, BEK, BFR-1, CD332, CEK3, CFD1, ECT1, JWS, K-SAM, KGFR, TK14, TK25
B5	FGFR3	NM_000142, NM_001163213, NM_022965	Fibroblast growth factor receptor 3	ACH, CD333, CEK2, HSFGFR3EX, JTK4
C5	FOS	NM_005252	FBJ murine osteosarcoma viral oncogene homolog	AP-1, C-FOS, p55
D5	FOXO1	NM_002015	Forkhead box O1	FKH1, FKHR, FOXO1A
E5	FOXO3	NM_001455, NM_201559	Forkhead box O3	AF6q21, FKHRL1, FKHRL1P2, FOXO2A, FOXO3
F5	GMPS	NM_003875	Guanine monphosphate synthase	
G5	HMGA2	NM_001300918, NM_001300919, NM_003483, NM_003484	High mobility group AT-hook 2	BABL, HMGI-C, HMGIC, LIPO, STQTL9
H5	IGF1	NM_000618, NM_001111283, NM_001111284, NM_001111285	Insulin-like growth factor 1	IGF-IA, IGFI, IGF1
A6	JAK2	NM_004972	Janus kinase 2	JTK10, THCYT3

B6	MAP2K1	NM_002755	Mitogen-activated protein kinase kinase 1	CFC3, MAPKK1, MEK1, MKK1, PRKMK1
C6	MDM2	NM_001145337, NM_001145339, NM_001145340, NM_001278462, NM_002392	MDM2 proto-oncogene, E3 ubiquitin protein ligase	ACTFS, HDMX, hdm2
D6	MET	NM_000245, NM_001127500	MET proto-oncogene, receptor tyrosine kinase	AUTS9, HGFR, RCCP2, c-Met
E6	KMT2A	NM_001197104, NM_005933	Lysine (K)-specific methyltransferase 2A	hCG_1732268, ALL-1, CXXC7, HRX, HTRX1, MLL, MLL/GAS7, MLL1, MLL1A, TET1-MLL, TRX1, WDSTS
F6	MMP2	NM_001127891, NM_001302508, NM_001302509, NM_001302510, NM_004530	Matrix metallopeptidase 2 (gelatinase A, 72kDa gelatinase, 72kDa type IV collagenase)	CLG4, CLG4A, MMP-2, MMP-II, MONA, TBE-1
G6	MMP9	NM_004994	Matrix metallopeptidase 9 (gelatinase B, 92kDa gelatinase, 92kDa type IV collagenase)	CLG4B, GELB, MANDP2, MMP-9
H6	MTA1	NM_001203258, NM_004689	Metastasis associated 1	
A7	MTA2	NM_004739	Metastasis associated 1 family, member 2	MTA1L1, PID
B7	MYC	NM_002467	v-myc avian myelocytomatosis viral oncogene homolog	MRTL, bHLHe39, c-Myc, MYCC
C7	NF1	NM_000267, NM_001042492, NM_001128147	Neurofibromin 1	NFNS, VRNF, WSS
D7	NF2	NM_000268, NM_016418, NM_181825, NM_181828, NM_181829, NM_181830, NM_181831, NM_181832, NM_181833	Neurofibromin 2 (merlin)	ACN, BANF, SCH
E7	NFKB1	NM_001165412, NM_003998	Nuclear factor of kappa light polypeptide gene enhancer in B-cells 1	EBP-1, KBF1, NF-kB1, NF-kappa-B, NF-kappaB, NFKB-p105, NFKB-p50, NFkappaB, p105, p50
F7	NFKBIA	NM_020529	Nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, alpha	IKBA, MAD-3, NFKBI
G7	NOTCH1	NM_017617	Notch 1	AOS5, AOVD1, TAN1, hN1
H7	NTRK1	NM_001007792, NM_001012331, NM_002529	Neurotrophic tyrosine kinase, receptor, type 1	MTC, TRK, TRK1, TRKA, Trk-A, p140-TrkA

A8	NTRK3	NM_001007156, NM_001012338, NM_001243101, NM_002530	Neurotrophic tyrosine kinase, receptor, type 3	TRKC, gp145(trkC)
B8	PDGFRA	NM_006206	Platelet-derived growth factor receptor, alpha polypeptide	CD140A, PDGFR-2, PDGFR2, RHEPDGFRA
C8	PDGFRB	NM_002609	Platelet-derived growth factor receptor, beta polypeptide	CD140B, IBGC4, IMF1, JTK12, PDGFR, PDGFR-1, PDGFR1
D8	PLAU	NM_001145031, NM_002658	Plasminogen activator, urokinase	RP11-417O11.1, ATF, BDPLT5, QPD, UPA, URK, u-PA
E8	PLAUR	NM_001005376, NM_001005377, NM_001301037, NM_002659	Plasminogen activator, urokinase receptor	CD87, U-PAR, UPAR, URKR
F8	PTEN	NM_000314	Phosphatase and tensin homolog	10q23del, BZS, CWS1, DEC, GLM2, MHAM, MMAC11, TEP1, PTEN
G8	RAF1	NM_002880	Raf-1 proto-oncogene, serine/threonine kinase	CMD1NN, CRAF, NS5, Raf-1, c-Raf
H8	RARA	NM_000964, NM_001024809, NM_001145301, NM_001145302	Retinoic acid receptor, alpha	NR1B1, RAR
A9	RB1	NM_000321	Retinoblastoma 1	RP11-174I10.1, OSRC, PPP1R130, RB, p105-Rb, pRb, pp110
B9	RET	NM_020630, NM_020975	Ret proto-oncogene	CDHF12, CDHR16, HSCR1, MEN2A, MEN2B, MTC1, PTC-ELE1, RET51, RET
C9	RUNX1	NM_001001890, NM_001122607, NM_001754	Runt-related transcription factor 1	AML1, AML1-EVI-1, AMLCR1, CBFA2, EVI-1, PEBP2aB
D9	SERPINB5	NM_002639	Serpin peptidase inhibitor, clade B (ovalbumin), member 5	PI5, maspin
E9	SERPINE1	NM_000602	Serpin peptidase inhibitor, clade E (nexin, plasminogen activator inhibitor type 1), member 1	PAI, PAI-1, PAI1, PLANH1
F9	SYK	NM_001135052, NM_001174167, NM_001174168, NM_003177	Spleen tyrosine kinase	p72-Syk
G9	TAL1	NM_001287347, NM_001290403, NM_001290404, NM_001290405, NM_001290406, NM_003189	T-cell acute lymphocytic leukemia 1	SCL, TCL5, bHLHa17, tal-1
H9	TERT	NM_001193376, NM_198253	Telomerase reverse transcriptase	CMM9, DKCA2, DKCB4, EST2, PFBMFT1, TCS1, TP2,

				TRT, hEST2, hTRT
A10	TFE3	NM_001282142, NM_006521	Transcription factor binding to IGHM enhancer 3	RCCP2, RCCX1, TFEA, bHLHe33
B10	TFEB	NM_001167827, NM_001271943, NM_001271944, NM_001271945, NM_007162	Transcription factor EB	RP4-696P19.3, ALPHATFEB, BHLHE35, TCFEB
C10	TGFB1	NM_000660	Transforming growth factor, beta 1	CED, DPD1, LAP, TGFB, TGFbeta
D10	TGFBR1	NM_001130916, NM_004612	Transforming growth factor, beta receptor 1	RP11-96L7.1, AAT5, ACVRLK4, ALK-5, ALK5, ESS1, LDS1, LDS1A, LDS2A, MSSE, SKR4, TGFR-1, tbetaR-I
E10	THBS1	NM_003246	Thrombospondin 1	THBS, THBS-1, TSP, TSP-1, TSP1
F10	TIMP1	NM_003254	TIMP metallopeptidase inhibitor 1	RP1-230G1.3, CLGI, EPA, EPO, HCI, TIMP
G10	TIMP2	NM_003255	TIMP metallopeptidase inhibitor 2	CSC-21K, DDC8
H10	TLX1	NM_001195517, NM_005521	T-cell leukemia homeobox 1	HOX11, TCL3
A11	TNF	NM_000594	Tumor necrosis factor	DADB-70P7.1, DIF-alpha, TNFA, TNFSF2, TNF
B11	TNFRSF10B	NM_003842, NM_147187	Tumor necrosis factor receptor superfamily, member 10b	UNQ160/PRO186, CD262, DR5, KILLER, KILLER/DR5, TRAIL-R2, TRAILR2, TRICK2, TRICK2A, TRICK2B, TRICKB, ZTNFR9
C11	TNFRSF1A	NM_001065	Tumor necrosis factor receptor superfamily, member 1A	CD120a, FPF, MS5, TBP1, TNF-R, TNF-R-I, TNF-R55, TNFAR, TNFR1, TNFR1-d2, TNFR55, TNFR60, p55, p55-R, p60
D11	TNFRSF25	NM_001039664, NM_003790, NM_148965, NM_148966, NM_148967, NM_148970	Tumor necrosis factor receptor superfamily, member 25	RP4-650H14.2, APO-3, DDR3, DR3, LARD, TNFRSF12, TR3, TRAMP, WSL-1, WSL-LR
E11	TP53	NM_000546, NM_001126112, NM_001126113, NM_001126114, NM_001126115, NM_001126116, NM_001126117, NM_001126118,	Tumor protein p53	BCC7, LFS1, P53, TRP53

		NM_001276695, NM_001276696, NM_001276697, NM_001276698, NM_001276699, NM_001276760, NM_001276761		
F11	TRADD	NM_003789	TNFRSF1A-associated via death domain	Hs.89862
G11	VEGFA	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	Vascular endothelial growth factor A	RP1-261G23.1, MVCD1, VEGF, VPF
H11	WT1	NM_000378, NM_001198551, NM_001198552, NM_024424, NM_024426	Wilms tumor 1	AWT1, EWS-WT1, GUD, NPHS4, WAGR, WIT-2, WT33

Introduction

Background

MIQE guidelines

MIQE (Minimum Information for Publication of Quantitative Real-Time PCR Experiments) guidelines (Bustin et al., 2009) are standard of real-time PCR (qPCR, quantitative PCR) experiments. When performing qPCR, it is very important to ensure that the procedures such as experimental design, implementation, and data analysis comply with the MIQE guidelines. MIQE guidelines are classified into 9 different parts (experimental design, sample, nucleic acid extraction, reverse transcription, qPCR target information, qPCR oligonucleotides, qPCR protocol, qPCR validation, data analysis), suggesting guidelines for the overall qPCR experiment. They aim to improve the reliability and reproducibility of qPCR experiments and results. *AccuPower*® qPCR Array System: Human Cancer qPCR panel kit has been developed expertise following the MIQE guidelines and provides accurate and reliable qPCR results of SCI publication grade.

Reverse transcription

RT-PCR must be carefully considered when optimizing the condition with specificity, sensitivity, reproducibility or fidelity of the reaction. Successful performance of the RT-PCR is dependent on a clear understanding of the primary aim of the assay. As RNA cannot serve as a template for PCR, the first step in the RT-PCR is the reverse transcription of the RNA template into cDNA, followed by its exponential amplification in the PCR.

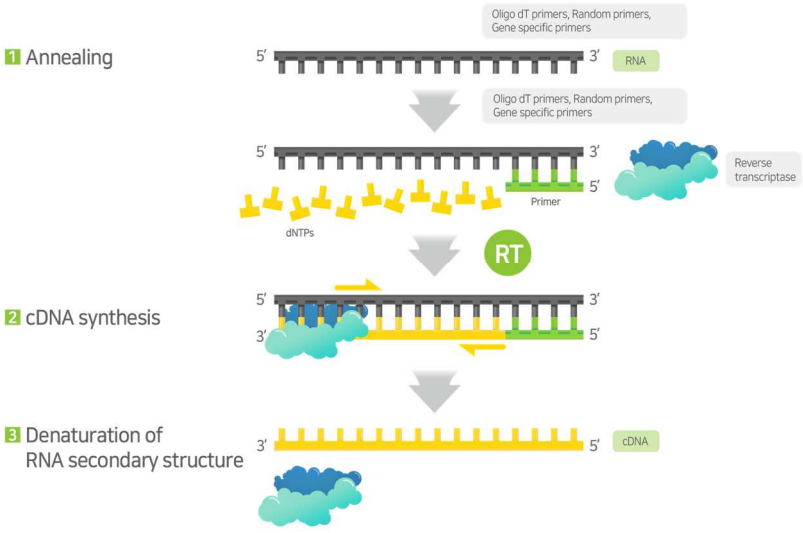

Figure 2. Procedures of reverse transcription.

The quantification of mRNA expression can be carried out by one-step or two-step RT-PCR. One-step RT-PCR in which RT reaction for cDNA synthesis and PCR for DNA amplification occur in a single test tube allows easy, fast result analysis with many samples. However, this

method is generally lower in accuracy and specificity than two-step RT-PCR and is not recommended for PCR with SYBR green that binds double stranded DNA and emits green light because of the possibility of primer dimer formation.

Two-step RT-PCR occurs in two steps of separate RT reaction and PCR. This method is more sensitive, and is useful in analyzing the expression of different target genes from a single sample by adjusting the amount of cDNA for the following reaction. It is also favored in the PCR using SYBR green due to its accuracy.

qPCR detection method

Intercalating dye-based method (dsDNA binding dye type)

Intercalating dye-base method is popular detection method in real-time PCR. Intercalating dye (ex. SYBR® Green) specifically binds to double-stranded DNA produced by DNA polymerase during PCR and emits fluorescence, resulting in an increase in fluorescence intensity up to a thousand times proportional to the amount of PCR products, which provides the basis for the mRNA quantification of target genes.

With intercalating dye-based method, there is no need to purchase expensive probes (e.g. TaqMan®), which reduces experimental costs and has the advantage of relatively simple primer design.

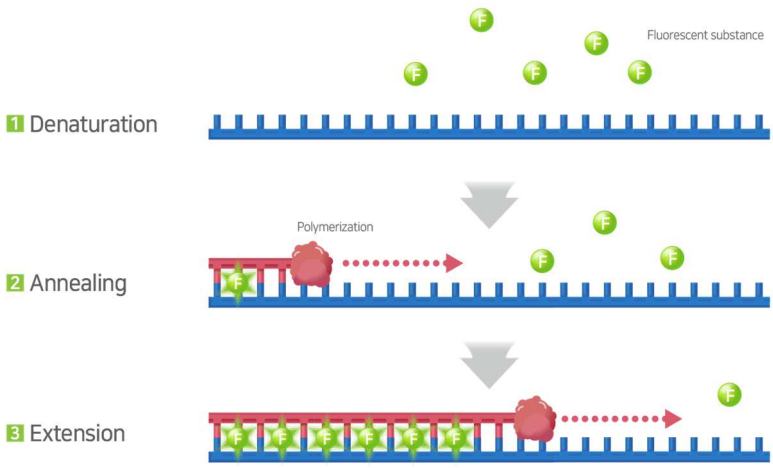

Figure 3. Overview of real-time PCR using intercalating dye-based method.

On the other hand, the greatest issue with intercalating dyes is their non-specificity. The dyes indiscriminately bind to all double-stranded DNA molecules not only target specific amplified PCR products but also non-specific artifacts (such as non-specific target or primer-dimer). Thus, well designed primers are necessary to avoid non-specific binding and primer dimerization. And melting curve analysis should be performed to exclude primer-dimer.

Primer design for qPCR

Primers of the human cancer panel are the product of BIONEER, which is one of the world's leading suppliers of synthetic oligonucleotides. There are several important points to design primers for qPCR on the basis of MIQE guidelines and all primers included in panel meet the followings.

Specific primer designing using primer Blast (NCBI) and BIONEER's bioinformatics tool.

- Designing primers longer than 19 bases.
- Specific dissociation curves.
- Detection of expected size with single band via gel electrophoresis.
- Short amplicon size (70-150 bp).

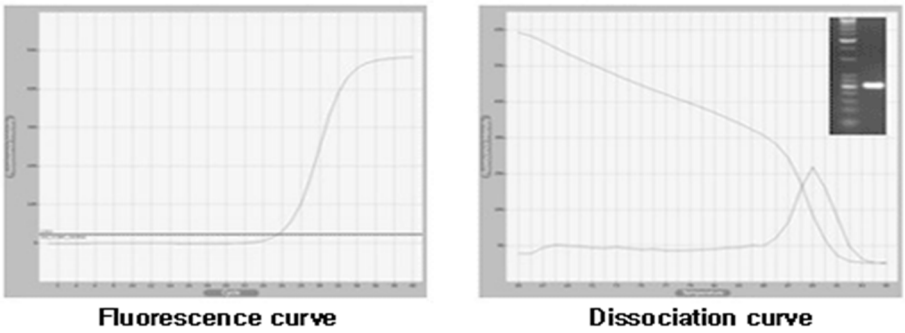

Figure 4. Primer validation result (e.g. CCND3 gene).

Product Description

The *AccuPower*® qPCR Array System: Human Cancer qPCR panel kit (96 well plate) is constructed in a panel format to screen genes involved in various cancer pathways or a specific cancer disease at once. Since all primers are designed and validated in accordance with the MIQE guidelines, the results can be used for SCI paper publication.

The human cancer panel contains 88 target genes which are related with apoptosis invasion & metastasis, angiogenesis, oncogenes & tumor suppressor, signal transduction & transcription factors, and cell cycle & DNA damage repair. This panel also contains 5 human reference genes of ACTB, B2M, GAPDH, HPRT1 and RPLP0 for relative quantification, and 3 control wells of non-template control (NTC), genomic DNA control (GDC), and positive PCR control (PPC).

All validated PCR primers for target genes and reference genes are coated in each well of the panel plates. The panel is designed to provide reproducibility and higher sensitivity in experiments along with significantly reducing non-specific reaction. It simplifies preparation of real-time PCR mixture by making the user add the template DNA, 2X Master Mix (intercalating dye type), and nuclease-free water only and users can get reliable data simply and conveniently.

Experimental Procedures

Start with isolating RNA from your experimental samples using *AccuPrep*® Universal RNA Extraction Kit (Cat. No. K-3141) or *MagListo*™ 5M Universal RNA Extraction Kit (Cat. No. K-3613). It is necessary to treat RNase-free DNase I to all qPCR samples. Then, convert the isolated RNA to cDNA template with *AccuPower*® *RocketScript*™ Cycle RT PreMix (dT_{20}) (Cat. No. K-2201). Add equal volume of the synthesized cDNA and *AccuPower*® 2X *GreenStar*™ qPCR Master Mix (Cat. No. K-6251) into each well of the sample PCR array plate containing the pre-dispensed gene-specific primer sets. And perform PCR with *Exicycler*™ 96 (Cat. No. A-2060-1). Use Analysis Exicycler4 software to calculate the threshold cycle (Ct) values for all the genes on each PCR array. Finally, calculate fold-changes in gene expression for pair-wise comparison using $2^{-\Delta\Delta Ct}$ method. It is the proper normalization method that the reference gene has consistency of Ct value on samples.

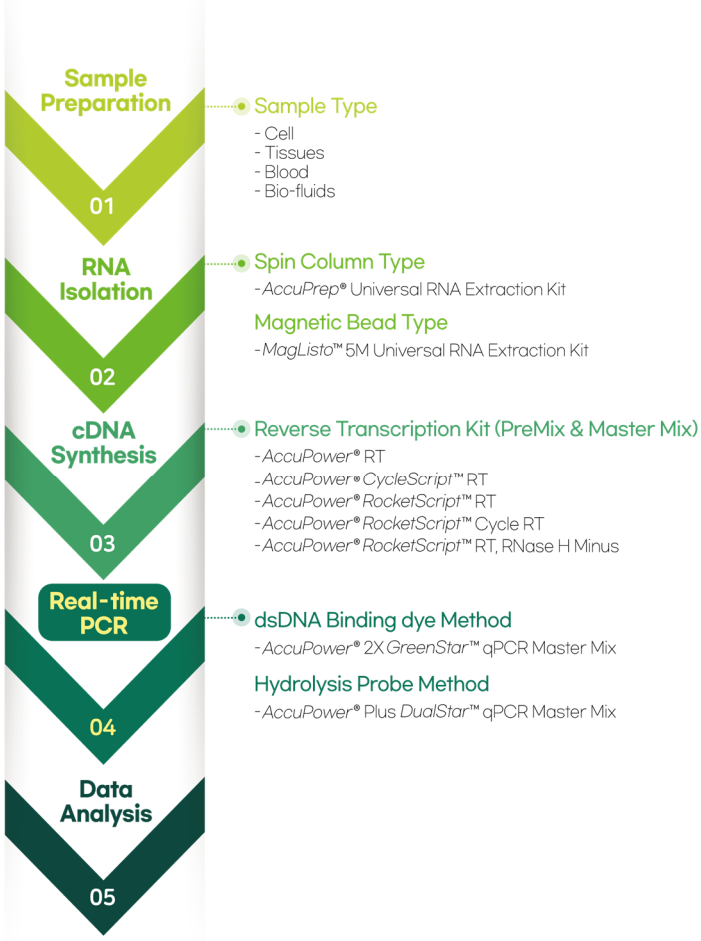

Figure 5. Schematic diagram of experimental procedures from sample preparation to data analysis.

Materials to be Prepared by Users

- *AccuPrep*® Universal RNA Extraction Kit (Cat. No. K-3141) or
  MagListo™ 5M Universal RNA Extraction Kit (Cat. No. K-3613)
- *AccuPower*® *RocketScript*™ Cycle RT PreMix (dT_{20}) (Cat. No. K-2201)
- *AccuPower*® 2X *GreenStar*™ qPCR Master Mix (Cat. No. K-6251)
- Real-time PCR instrument [*Exicycler*™ 96 (Cat. No. A-2060-1)]
- Nuclease-free water
- 96-100% ethanol

RNA Isolation with Spin Column Type

AccuPrep® Universal RNA Extraction Kit is designed for extraction of highly purified RNA from cultured cells, plant and animal tissues within 20 min. The kit employs *AccuPrep*® Binding Column-III with silica membrane for nucleic acid binding in the presence of chaotropic salts. This silica membrane has enough surface area to bind up to 120 µg of RNA. The process does not require phenol/chloroform extraction, ethanol precipitation, or other time-consuming steps. RNA extracted through this kit can be used for a variety of applications, including reverse transcription PCR (RT-PCR), reverse transcription quantitative PCR (RT-qPCR), Northern blot analysis, and cDNA synthesis. We recommend DNase treatment for only RNA quantitation.

Specifications

***AccuPrep*® Universal RNA Extraction Kit**		
Amount of Starting Sample	Cultured cells	10^4-10^8 cells
	Animal tissue	25-50 mg
Typical RNA Yield	Cultured cells	15-20 µg
	Animal tissue	10-60 µg
Column Binding Capacity		Up to 120 µg
Column Loading Volume		800 µl
Elution Volume		30-100 µl
RNA Purity		A_{260}/A_{280} > 2.0, A_{260}/A_{230} > 1.7
Isolation Technology		Silica Column

*** Note:** There may be differences in measured values depending on the type of samples.

Before You Begin

Before proceeding, please check the following:

1. Add 10 µl of β-mercaptoethanol per 1 ml of RB Buffer.

2. g-force can be calculated as follows: rcf = 1.12 x r x $(rpm/1,000)^2$

   *** Note:** Where 'rcf' is the relative centrifugal force (in g), 'r' is the radius of the rotor in centimeters, and rpm is the speed of the centrifuge in revolutions per minute.

Preparing Lysates from Animal Tissue

1. **(Lysis & homogenization)** Grind (or homogenize) 20-30 mg of fresh or thawed animal tissue sample with a mortar and pestle (or homogenizer) and place it into a clean 1.5 ml tube.

2. Add 500 µl of RB Buffer to the sample and mix thoroughly by vortexing.

3. Centrifuge at full speed for 3 minutes and carefully transfer the supernatant to a new 1.5 ml tube.

   *** Note: (Optional)** Centrifugation through the *AccuPrep*® Filtering Column (Cat. No. KA-1160) removes debris effectively.

4. **(RNA precipitation)** Add 200 µl of absolute ethanol (not provided) to the lysate and mix immediately by pipetting.

   *** Note:** Do not centrifuge.

5. Proceed immediately to "Purification Procedure Using Spin Columns" on page 19.

Preparing Lysates from Cultured Cells

1. **(Cell harvesting)** Harvest cells according to step 1-A or 1-B.

1-A. Suspension cell culture:

Harvest cultured cells (10^4-10^6 cells) by centrifugation at 300 x *g* for 5 minutes to pellet cells. Discard the supernatant carefully without disturbing a cell pellet and go to step 2.

1-B. Monolayer cell culture:

There are two different methods to collect cells grown in monolayer culture.

a. Direct cell harvesting on the culture dishes:
   Completely discard the cell culture medium and go to step 2.
   * **Note**: You should completely remove the cell culture medium because it may inhibit the RNA extraction.

b. Cell harvesting with trypsin:
   Remove the cell culture medium and wash the cell monolayer with DPBS. Add 0.1-0.25% trypsin to the washed cell monolayer to detach the cells. After the cells detach, add cell culture medium. Transfer the cells to an RNase-free tube (not provided) and centrifuge at 300 x *g* for 5 minutes. Discard the supernatant carefully and go to step 2.

2. **(Lysis & homogenization)** Resuspend the cell pellet from step 1 in 400 µl of RB Buffer by vortexing.
   * **Note:** You should completely resuspend the sample to achieve maximum lysis efficiency.

3. **(RNA precipitation)** Add 300 µl of 80% ethanol† to the lysate and mix immediately by pipetting.
   * **Note:** Do not centrifuge.
   † When purifying RNA from certain cell lines, precipitates may be visible after addition of ethanol. This does not affect the procedure.

4. Proceed immediately to “Purification Procedure Using Spin Columns” on page 19.

Purification Procedure Using Spin Columns

1. **(RNA binding)** Transfer up to 700 µl of sample to the *AccuPrep*® Binding Column-III fit in a 2 ml collection tube. Close the lid gently and centrifuge at ≥14,000 rpm for 20 seconds. Discard the flow through†. Reuse the collection tube in step 2.
   * **Note:** If the sample volume exceeds 700 µl, centrifuge successive aliquots in the same *AccuPrep*® Binding Column-III and discard the flow through.
   † Discard the flow through after each centrifugation.

2. **(1st Washing)** Wash the *AccuPrep*® Binding Column-III by adding 700 µl of RWA1 Buffer.

3. Close the lid gently and centrifuge at 14,000 rpm for 20 seconds. Discard the flow through. Reuse the collection tube in step 4.
   * **Note:** After centrifugation, carefully remove the *AccuPrep*® Binding Column-III from the collection tube so that the column does not contact the flow through.

4. **(2nd Washing)** Wash the *AccuPrep*® Binding Column-III by adding 500 µl of RWA2 Buffer.

5. Close the lid gently and centrifuge at 14,000 rpm for 20 seconds. Discard the flow through. Reuse the collection tube in step 6.

6. Wash the *AccuPrep*® Binding Column-III by adding 500 µl of RWA2 Buffer.

7. Close the lid gently and centrifuge at 14,000 rpm for 2 minutes. Discard the flow through. Reuse the collection tube in step 8. The long centrifugation dries the spin column membrane, ensuring that no ethanol is carried over during RNA elution.
   * **Note:** Residual ethanol may interfere with downstream reactions. After centrifugation, carefully remove the *AccuPrep*® Binding Column-III from the collection tube so that the column does not contact the flow through.

8. Centrifuge once more at 14,000 rpm for 1 minute to remove residual ethanol completely and check whether there is no droplet clinging to the bottom of the binding column.

9. **(Elution)** Place the *AccuPrep*® Binding Column-III in a clean 1.5 ml Tube (supplied with a kit). Add 50-200 µl of ER Buffer or RNase-free water to elute RNA.

10. Incubate at room temperature for 1 minute to be absorbed the ER Buffer completely into the glass fiber of the binding column.

11. Close the lid gently and centrifuge at 10,000 rpm for 1 minute.

12. To recover more RNA (>30 μg), repeat once more elution step using the eluate from step 11.

13. Close the lid gently and centrifuge at 10,000 rpm for 1 minute.

14. **(Note)** DNase treatment must be performed on the sample to confirm accurate mRNA expression.

RNA Clean-Up

1. Adjust the sample to a volume of 100 µl with RNase-free water. Add 400 µl RB Buffer and mix well.

2. Add 300 µl of 80% ethanol to the diluted RNA and mix well by pipetting.
   * **Note:** Do not centrifuge.

3. Transfer the sample to the *AccuPrep*® Binding Column-III fit in a 2 ml collection tube. Close the lid gently and centrifuge at ≥14,000 rpm for 20 seconds. Discard the flow through†. Reuse the collection tube in step 4.
   * **Note:** After centrifugation, carefully remove the *AccuPrep*® Binding Column-III from the collection tube so that the column does not contact the flow through.
   † Discard the flow through after each centrifugation.

4. Wash the *AccuPrep*® Binding Column-III by adding 500 µl of RWA2 Buffer.

5. Close the lid gently and centrifuge at 14,000 rpm for 2 seconds. Discard the flow through. Reuse the collection tube in step 6.

6. Wash the *AccuPrep*® Binding Column-III by adding 500 µl of RWA2 Buffer.

7. Close the lid gently and centrifuge at 14,000 rpm for 2 minutes. Discard the flow through. Reuse the collection tube in step 8. The long centrifugation dries the spin column membrane, ensuring that no ethanol is carried over during RNA elution.

8. Centrifuge once more at 14,000 rpm for 1 minute to remove residual ethanol completely and check whether there is no droplet clinging to the bottom of the binding column.

9. Place the *AccuPrep*® Binding Column-III in a clean 1.5 ml Tube (supplied with a kit). Add 50-200 µl of ER Buffer or RNase-free water to elute RNA.

10. Incubate at room temperature for 1 minute to be absorbed the ER Buffer completely into the glass fiber of the binding column.

11. Close the lid gently and centrifuge at 10,000 rpm for 1 minute.

RNA Isolation with Magnetic Bead Type

MagListo™ 5M Universal RNA Extraction Kit is designed for extraction of highly purified RNA from cultured cells, plant and animal tissues. The kit employs Magnetic Nano Beads to extract total RNA with the aid of *MagListo*™ Magnetic Separation Rack and *ExiPrep*™ 96 Lite (Cat. No. A-5250). The use of *MagListo*™ Magnetic Separation Rack along with the kit greatly increases user convenience by shortening the extraction time without centrifugation. On the same principle, *ExiPrep*™ 96 Lite is designed for rapid extraction of nucleic acids delivering up to 96 extracted samples (including controls).

RNA extracted through this kit can be used for a variety of applications, including: reverse transcription PCR (RT-PCR), reverse transcription quantitative PCR (RT-qPCR), northern blot analysis, and cDNA synthesis.

Specifications

***MagListo*™ 5M Universal RNA Extraction kit**			
Amount of Starting Sample	Cultured cells		10^4-10^8 cells
	Liver		25-50 mg
	Spleen		100 mg
Typical RNA Yield	Cultured cells		15-20 µg
	Liver		10-60 µg
	Spleen		30-60 µg
Typical RNA Yield	Scale	Mini	up to 100 µg
		Midi	up to 500 µg
Turnaround Time		Mini	< 10 min
		Midi	< 15 min
Elution Volume		Mini	50 µl
		Midi	500 µl
RNA Purity			A_{260}/A_{280} > 2.0, A_{260}/A_{230} > 1.7
Isolation Technology			Magnetic Nano Bead

*** Note:** There may be differences in measured values depending on the type of samples.

Before You Begin

Before proceeding, please check the following:

1. Add 10 µl of β-mercaptoethanol per 1 ml of RD Buffer.

2. g-force can be calculated as follows: rcf = 1.12 x r x $(rpm/1,000)^2$

   *** Note:** Where 'rcf' is the relative centrifugal force (in g), 'r' is the radius of the rotor in centimeters, and rpm is the speed of the centrifuge in revolutions per min.

Preparing Lysates from Animal Tissue

1. **(Lysis & homogenization)** Grind (or homogenize) 20-30 mg (mini)* of fresh or thawed animal tissue sample with a mortar and pestle (or homogenizer) and place it into appropriate tubes.

   *** Note:** The amount of sample required may vary depending on the extraction scale.

2. Add 500 µl (mini) / 5 ml (midi) of RD Buffer to the sample and mix thoroughly by vortexing.

3. Centrifuge at full speed for 3 minutes and carefully transfer the supernatant to new 1.5 ml tubes (mini) or 50 ml tubes (midi) with a pipette.

4. **(RNA precipitation)** Add 300 µl (mini) / 3 ml (midi) of absolute ethanol (not provided) to the lysate and mix immediately by pipetting.

   *** Note:** Do not centrifuge.

5. Proceed immediately to "Purification Procedure Using Magnetic Nano Beads" on page 25.

Preparing Lysates from Cultured Cells

1. **(Cell harvesting)** Harvest cells according to step 1-A or 1-B.

1-A. Suspension cell culture:

Harvest cultured cells (10^4-10^6 cells, mini)* by centrifugation at 300 x *g* for 5 minutes to pellet cells. Discard the supernatant carefully without disturbing a cell pellet and go to step 2.

*** Note:** The amount of sample required may vary depending on the extraction scale.

1-B. Monolayer cell culture:

There are two different methods to collect cells grown in monolayer culture.

a. Direct cell harvesting on the culture dishes:
   Completely discard the cell culture medium and go to step 2.
   *** Note**: You should completely remove the cell culture medium because it may inhibit the RNA extraction.

b. Cell harvesting with trypsin:
   Remove the cell culture medium and wash the cell monolayer with DPBS. Add 0.1-0.25% trypsin to the washed cell monolayer to detach the cells. After the cells detach, add cell culture medium. Transfer the cells to an RNase-free tube (not provided) and centrifuge at 300 x *g* for 5 minutes. Discard the supernatant carefully and go to step 2.

2. **(Lysis & homogenization)** Resuspend the cell pellet from step 1 in 500 µl (mini) / 5 ml (midi) of RD Buffer by vortexing.
   *** Note:** You should completely resuspend the sample to achieve maximum lysis efficiency.

3. **(RNA precipitation)** Add 300 µl (mini) / 3 ml (midi) of absolute ethanol† (not provided) to the lysate and mix immediately by pipetting.
   *** Note:** Do not centrifuge.
   † When purifying RNA from certain cell lines, precipitates may be visible after addition of ethanol. This does not affect the procedure.

4. Proceed immediately to “Purification Procedure Using Magnetic Nano Beads” on page 25.

Purification Procedure Using Magnetic Nano Beads

1. **(RNA binding)** Add 100 µl (mini) / 1 ml (midi) of Magnetic Nano Bead solution to each tube and mix thoroughly by vortexing until the beads are fully resuspended.

2. Place the tube in *MagListo*™-2 (mini) or *MagListo*™-50 (midi) Magnetic Separation Rack with the magnet plate attached and invert the rack gently 3-4 times until the beads bind tightly to the magnet.

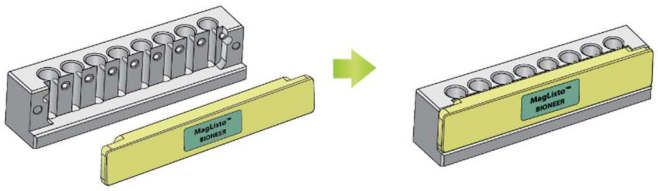

Figure 6. Attachment of the magnet plate. Combine the magnet plate to the stand.

3. Without removing the tube from *MagListo*™ Magnetic Separation Rack, discard the supernatant carefully and completely remove the remaining supernatant using a paper towel by blotting. During this process, the beads containing RNA remain attached to the side of the tube.

   * **Note:** If you want to perform the optional RNA Clean-Up, follow the steps on page 28 after performing this step.

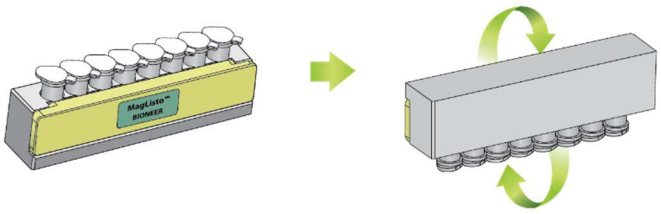

Figure 7. How to discard the supernatant. Discard the supernatant by inverting the *MagListo*™ Magnetic Separation Rack. The silicone immobilizer inside the stand prevents the tubes from falling. When discarding the supernatant, invert the rack completely so that the solution does not spill on the rack.

4. **(1st Washing)** Detach the magnet plate from *MagListo*™ Magnetic Separation Rack. Add 800 µl (mini) or 8 ml (midi) of RWM1 Buffer to each tube and close the cap. Mix by vortexing until the beads are fully resuspended.

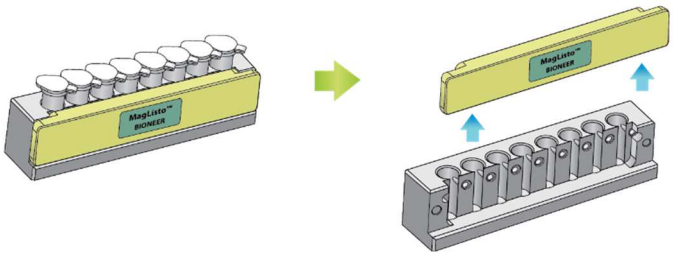

Figure 8. Detachment of the magnet plate. Pull the magnet plate up and gently separate it.

5. Attach the magnet plate to stand and invert the rack gently 3-4 times until the beads bind tightly to the magnet.

6. Without removing the tubes from *MagListo*™ Magnetic Separation Rack, discard the supernatant carefully and completely remove the remaining supernatant using a paper towel by blotting.

7. **(2nd Washing)** Repeat steps 4-6 by adding 800 µl (mini) or 8 ml (midi) of RWA2 Buffer for additional washing. Repeat steps 5-6 once more.

8. **(3rd Washing)** Remove residual ethanol according to step 8-A or 8-B.

8-A. Washing beads:

Without removing the tube from *MagListo*™ Magnetic Separation Rack, add 700 µl (mini) or 10 ml (midi) of WE Buffer to the opposite side of beads. Close the cap and invert the rack twice in order to remove ethanol from the sample. Discard the supernatant carefully and completely remove the remaining supernatant using a paper towel by blotting.

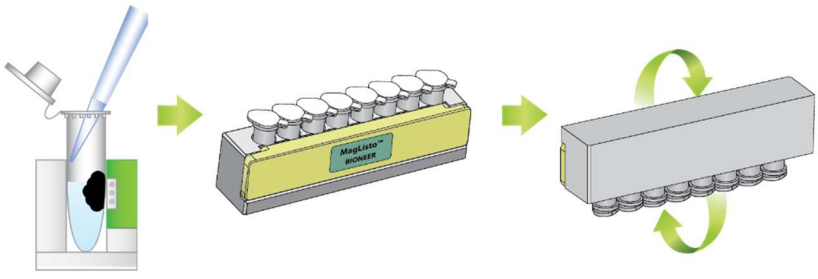

Figure 9. Washing the beads to remove residual ethanol. Please refer to the information above.

*** Note:** Direct pipetting of WE Buffer onto the beads, vortexing and/or vigorous shaking of the tubes may

release nucleic acid from the beads, which may result in lower RNA yield.

8-B. Drying beads:
Add 800 µl of 80% ethanol, mix thoroughly by vortexing, and repeat the steps 5-6. Completely dry the beads with the tube open at 60°C for at least 5 minutes. Remove the remaining supernatant with a pipette.

9. **(Elution)** Detach the magnet plate from *MagListo*™ Magnetic Separation Rack. Add 50-100 µl (mini) or 500 µl-1 ml (midi) of ER Buffer to each tube and resuspend RNA by vortexing or pipetting.

10. Incubate at 55-65°C for 1 minute.

11. Attach the magnet plate to *MagListo*™ Magnetic Separation Rack and invert the rack gently 3-4 times until the beads bind tightly to the magnet.

12. Without removing the tube from *MagListo*™ Magnetic Separation Rack, transfer supernatant containing RNA carefully to a new tube.

13. Discard the tubes with the remaining beads.
*** Note:** Do not reuse the beads.

14. **(Note)** DNase treatment must be performed on the sample to confirm accurate mRNA expression.

RNA Clean-Up

1. Adjust the sample to a volume of 100 µl with RNase-free water.

   *** Note:** If DNA-free RNA is required, add RNase-free DNase and DNase reaction buffer to each tube and adjust the volume up to 100 µl with RNase-free water. Incubate at room temperature for 10 min.

2. Add 100 µl of RD Buffer and mix well by pipetting.

3. Add 200 µl of absolute ethanol and mix well by pipetting.

4. Add 100 µl of Magnetic Nano Beads and mix well by pipetting until the beads are fully resuspended.

*** Note:** Please mix well Magnetic Nano Beads by vortexing before use.

5. Place the tube in *MagListo*™-2 (mini) Magnetic Separation Rack with the magnet plate attached and invert the rack gently 3-4 times until the beads bind tightly to the magnet.

6. Without removing the tube from *MagListo*™ Magnetic Separation Rack, discard the supernatant carefully and completely remove the remaining supernatant using a paper towel by blotting.

7. Go to step 4 of Purification Procedure Using Magnetic Nano Beads on page 25.

Reverse Transcription

1. Add template RNA (0.4-1 μg for total RNA) and nuclease-free water into *AccuPower*® *RocketScript*™ Cycle RT PreMix (dT_{20}) (Cat. No. K-2201, not provided) tubes to make a total volume of 20 μl or 50 μl. Do not include the dried pellet.

2. Dissolve the vacuum-dried pellet by vortexing or pipetting, and briefly spin down.

3. Perform the reaction under the following conditions.

1) CTRT reaction (Example 1)

Step	Temperature	Time	Cycles
Primer annealing	37°C	10-30 sec	10 cycles or more
cDNA synthesis	50°C	4 min	
Melting secondary structure & cDNA synthesis	55-60°C	30 sec	
Heat inactivation	95°C	5 min	1 cycle

2) CTRT reaction (Example 2)

Step	Temperature	Time	Cycles
Primer annealing	37°C	1 min	10 cycles or more
Melting secondary structure & cDNA synthesis	42-70°C	4 min	
Heat inactivation	95°C	5 min	1 cycle

3) Single temperature reaction (Example 3)

Step	Temperature	Time	Cycles
cDNA synthesis	22-55°C*	30-60 min	1 cycle
Heat inactivation	95°C	5 min	1 cycle

*** Note:** Recommended temperature is range of 42-48°C.

4. After the reaction, maintain the reaction mixture at 4°C. The samples can be stored at -20°C until use.

Real-time PCR

1. Prepare template DNA, *AccuPower*® 2X *GreenStar*™ qPCR Master Mix (K-6251, not provided), and nuclease-free water in a tube to make a total volume of 50 µl as described in following table.

Components	50 µl reaction
AccuPower® 2X *GreenStar*™ qPCR Master Mix	25 µl
Template DNA	5 pg-100 ng
(Optional) 80X ROX dye	0.625-5 µl
Nuclease-free water	Variable
Total volume	50 µl

*** Note:** ROX dye is used for normalization of intensity by background subtraction. The use of ROX dye is recommended for Applied Biosystems 7500 Real-Time PCR System, but not required for BIONEER *Exicycler*™ 96 Real-Time PCR System.

2. Carefully remove the covered film of panel and dispense 50 µl of reaction mixture into each well of *AccuPower*® qPCR Array System: Human Cancer qPCR panel kit.

*** Note:** Change pipette tips following each pipetting step to avoid cross-contamination among the wells.

3. Seal the plate with adhesive optical sealing film (Cat. No. 3111-4110) and briefly spin down.

4. Then, completely mix by vortexing to resuspend lyophilized primers and spin down again.

*** Note:** Before start, check carefully if there are residues on the film.

5. Perform the reaction under the following conditions.

Step	Temperature	Time	Cycles
Pre-denaturation	95°C	10 min	1 cycle
Denaturation	95°C	5 sec	40 cycles
Annealing	58°C	25 sec	
Extension	72°C	30 sec	
Detection	Scan		
Final extension	65°C	5 min	1 cycle
Melting	65-95°C	1 sec	-

6. After the reaction, perform data analysis.

Data Analysis

Two most commonly used methods to analyze data from qPCR are absolute quantification and relative quantification. Absolute quantification determines the input copy number, usually by calculating the PCR signal on the basic of a standard curve. Relative quantification relates the PCR signal of the target transcript in a treatment sample to that of an untreated control sample. The $2^{-\Delta\Delta Ct}$ method is a reasonable way to analyze the relative changes in gene expression from real-time quantitative PCR (qPCR) experiments. For our Human cancer panel is suitable for analysis by the $2^{-\Delta\Delta Ct}$ method.

- $\Delta Ct = Ct_{[target\ gene]} - Ct_{[reference\ gene]}$
- $\Delta\Delta Ct = \Delta Ct_{[treated\ sample]} - \Delta Ct_{[control\ sample]}$
- Fold Change = $2^{-\Delta\Delta Ct}$

- ΔCt: the difference between Ct value of target gene and Ct value of reference gene.
- $\Delta\Delta Ct$: the difference between average Ct value of treated sample and average of Ct value of control sample.
- $2^{-\Delta\Delta Ct}$: fold change in gene expression of the treated sample compared to the untreated control sample.

References

Bustin, S. A. (2000). Absolute quantification of mRNA using real-time reverse transcription polymerase chain reaction assays. *Journal of molecular endocrinology*, *25*(2), 169-193.

Bustin, S. A., Benes, V., Garson, J. A., Hellemans, J., Huggett, J., Kubista, M., ... & Wittwer, C. T. (2009). The MIQE Guidelines: Minimum Information for Publication of Quantitative Real-Time PCR Experiments.

Livak, K. J., & Schmittgen, T. D. (2001). Analysis of relative gene expression data using real-time quantitative PCR and the 2− ΔΔCT method. *methods*, *25*(4), 402-408.

Ordering Information

Description		Cat. No
AccuPower® qPCR Array System: Human Cancer qPCR panel kit	88 genes	S-6042-PH1

Related Products

Description		Cat. No
AccuPrep® Universal RNA Extraction Kit		K-3141
MagListo™ 5M Universal RNA Extraction Kit		K-3613
MagListo™-2 Magnetic Separation Rack		TM-1010
MagListo™-50 Magnetic Separation Rack		TM-1030
AccuPower® *RocketScript*™ Cycle RT PreMix	dT_{20}	K-2201
	dN_6	K-2205
	dN_{12}	K-2208
AccuPower® 2X *Greenstar*™ qPCR Master Mix		K-6251
ExiPrep™ 96 Lite		A-5250
AllInOneCycler™ PCR system		A-2041
Exicycler™ 96		A-2060-1
AccuPower® qPCR Array System: Single gene qPCR Primer Set		S-6042-S200

Explanation of Symbols

 Caution	 Consult Instructions For Use	 Do not Re-use	 Use-by Date

BIONEER Corporation - HQ

Address 8-11 Munpyeongseo-ro, Daedeok-gu, Daejeon, 34302, Republic of Korea
E-mail sales@bioneer.co.kr
Web www.bioneer.com

BIONEER Global Center

Address 71, Techno 2-ro, Yuseong-gu, Daejeon, 34013, Republic of Korea
E-mail sales@bioneer.co.kr
Web www.bioneer.com

BIONEER R&D Center

Address Korea Bio Park BLDG #B-702, 700 Daewangpangyo-ro, Bundang-gu, Seongnam-si Gyeonggi-do, 13488, Republic of Korea
E-mail sales@bioneer.co.kr
Web www.bioneer.com

BIONEER Inc. - USA Branch

Address 155 Filbert St. Suite 216 Oakland, CA 94607, USA
E-mail order.usa@bioneer.com
Web us.bioneer.com

BIONEER Corp. - European Branch

Address Ludwig-Erhard-Strasse 30-34, 65760 Eschborn, Germany
E-mail euinfo@bioneer.com
Web www.bioneer.com

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