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EcoQprep™ mRNA Kit

- 50 rxn

K-3708

Name	Volume	Quantity	Name	Volume	Quantity
MRD Buffer	30 mL	1 ea	1.5mL Tube	50 ea	1 pack
MRW1 Buffer	40 mL	1 ea	One Page Protocol	-	1 ea
MRW2 Buffer	50 mL	1 ea			
MRW3 Buffer	50 mL	1 ea			
EM Buffer	25 mL	1 ea			
Magnetic Nano Bead-Oligo dT	3 mL	1 ea			

Before Start

Components	Before Use	Storage	Symbol
MRD Buffer	Before each experiment, mix MRD buffer with 1 µL of β-mercaptoethanol per 1 mL of MRD buffer.	Do not store the prepared solution; freshly prepare it for each experiment.	 Run centrifuge  Heating  Caution  Add the following solution
All reagents	MRD Buffer, MRW1 Buffer, MRW2 Buffer, MRW3 Buffer, EM Buffer, Magnetic Nano Bead-Oligo dT	After opening, store at 2-8°C	

Protocol

NOTE Do not vortex after adding the solution; mix by pipetting or inverting instead.

Sample		Pre-treatment & Lysis	1	Bead Wash	2	Binding	3	1 st Wash	4	2 nd Wash	5	3 rd Wash	6	Elution	7
Cultured cells	Suspension cell culture	Cell pellet: ~1x10 ⁶ cells ☑ 300 xg, 5 min ⊕ 300 μL MRD with β-mercaptoethanol	⊕ 50 μL Bead to a new tube. ⊕ 50 μL MRD with β-mercaptoethanol	1) Attach magnet		⊕ the Lysate after lysis 1) Attach magnet		⊕ 600 μL MRW1 1) Attach magnet		⊕ 300 μL MRW2 1) Attach magnet		⊕ 700 μL MRW3 ❗ Add to the Opposite side of the bead ❗ Do Not Vortex 1) Gently Invert 3~4X		⊕ 15 - 20 μL EM 🔥 65°C, 2 min 1) Attach magnet	
	Monolayer cell culture	A) Direct harvest Discard cell culture medium ⊕ 300 μL MRD with β-mercaptoethanol B) Trypsin harvest Detach cells ☑ 300 xg, 5 min ⊕ 300 μL MRD with β-mercaptoethanol	1) Attach magnet  2) Discard sup 		2) Invert 3~4X  3) Discard sup 		2) Invert 3~4X  3) Discard sup 		3) Discard sup  4) Detach magnet 		2) Discard sup  3) Detach magnet 		2) Transfer the eluent  ❗ Immediately transfer the eluent to a new tube.		
Plant tissue		Tissue : ~20 mg Grind in LN ₂ ⊕ 300 μL MRD with β-mercaptoethanol per 10 mg tissue ☑ full speed, 3 min Transfer the supernatant	3) Detach magnet 		4) Detach magnet 		4) Detach magnet 		⊕ 300 μL MRW2 Repeat 1) ~ 3) ❗ Do Not Detach magnet at the final step						
Animal tissue		Homogenize sample : ~10 mg ⊕ 300 μL MRD with β-mercaptoethanol ☑ full speed, 3 min Transfer the supernatant	⊕ 50 μL MRD with β-mercaptoethanol Repeat 1) ~ 3)												