RIG-1 dsRNA Detection Kit

Cat.No.:HYJ522

Product Description

Retinoic acid-inducible gene I (RIG-I) functions as a cytosolic pattern recognition receptor that initiates innate antiviral immunity by detecting exogenous viral RNAs. Structurally, RIG-I is comprised of three main functional domains –Caspase Activation and Recruitment Domain (CARD) for signal transduction, DExD/ H-box RNA helicase domain, and C-terminal domain for RNA binding. The RNA helicase domain is associated with RNA-dependent ATP hydrolysis activities, which is activated upon binding to double-stranded RNA (dsRNA), leading to the hydrolysis of ATP into ADP and inorganic phosphate (Pi). This kit quantifies dsRNA content in samples by measuring ADP production during the reaction between RIG-I and the sample. The assay is performed in a multi-well plate and can detect reactions in volumes as low as 5 µL.The detection involves three steps:

1. RIG-I Reaction: Mix RIG-I enzyme, ATP, and the sample, followed by incubation.
2. ATP Depletion: Add an equal volume of Reagent 1 to deplete residual ATP.
3. ADP Detection: Add Reagent 2 to convert ADP back to ATP, which is then quantified using a luciferase/luciferin reaction. The luminescent signal is measured with a plate reader. dsRNA content is calculated based on a standard curve.

Components

	Components	96 T	384 T
RIG-I ATPase Reaction	RIG-I enzyme（1µM）	100µL	400µL
	10× RIG-I Reaction Buffer	0.25mL	1mL
	1M DTT	20µL	50µL
	10mM ATP	25µL	100µL
	Standard (UTP, 25ng/µL)	40µL	150µL
	Standard (N1-Me-pUTP,25ng/µL)	40µL	150µL
Measurement of ADP	Reagent 1-Assay buffer	0.5mL	2mL
	Reagent 1-Enzyme(5×)	0.125mL	0.5mL
	Reagent 1-0.5M $MnCl_2$	25µL	100µL
	Reagent 2	1.25mL	5mL
	384-well Plate	1 plate	1 plate

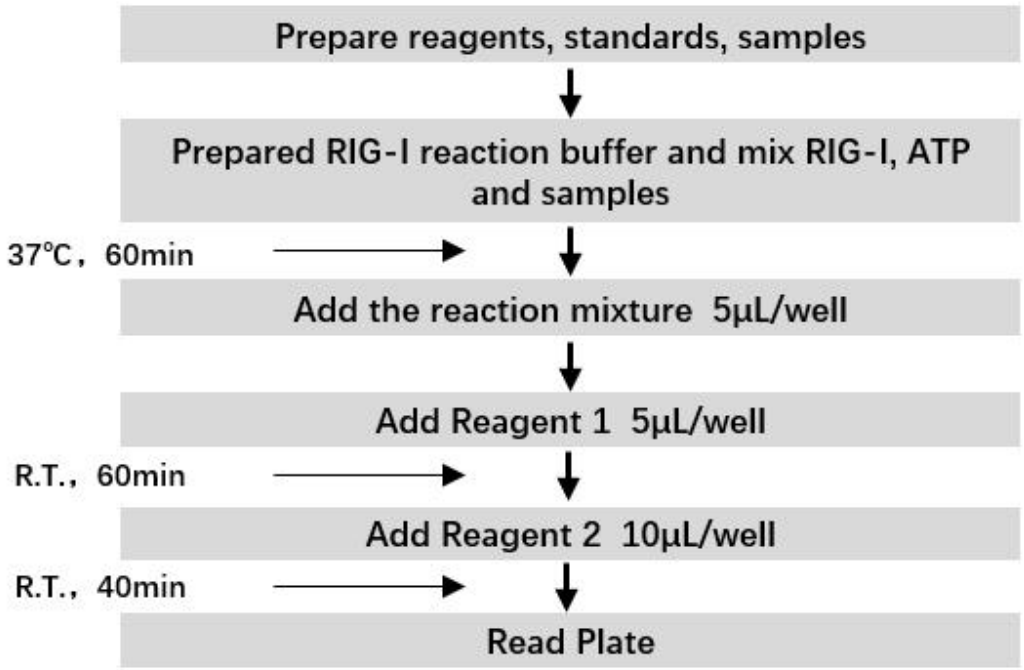
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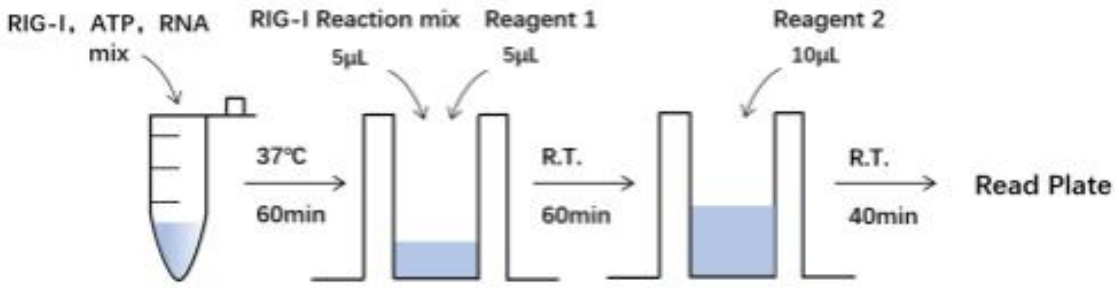
1. Dry Ice Shipping. Store the system at -25~- 15℃.
2. Before use, all components should be thawed and mixed thoroughly at room temperature. Once prepared, dispense all reagents into aliquots and store at -25~-15℃.Prepare the RIG-I system and Reagent 1 fresh for each use. Reagent 2 can be placed at room temperature (18–25°C) for 24 hours without significant signal loss.

Additional Materials Required

1. Chemiluminescence microplate reader.
2. Microplate shaker.
3. Handheld centrifuge.
4. Micropipette.
5. RNase-free tips and centrifuge tubes.

Operation Flowchart





Instructions

1. Preparation before the experiment

1) Thaw all reagents to room temperature (18~25°C) and mix thoroughly.

2) Avoid repeated freeze-thaw cycles. Aliquot unused reagents and store at -25~-15°C.

3) Prepare 1× RIG-I Reaction Buffer: For 1 mL, mix 895 µL RNase-free water, 100 µL 10× RIG-I Reaction Buffer, and 5 µL 1M DTT. Final concentrations: 1× buffer, 5 mM DTT.

4) Prepare Reagent 1: For 100 µL, mix 79 µL Reagent 1-Assay buffer, 20 µL 5× Reagent 1- Enzyme, and 1 µL 0.5 M $MnCl_2$. Final concentrations: 1× enzyme, 5 mM $MnCl_2$.

2. Detection steps

1) Thaw all reagents to room temperature (18~25°C).

2) Prepare 1× RIG-I Reaction Buffer (example for 1 mL):

Components	Volume
10× RIG-I Reaction Buffer	100 µL
1M DTT	5 µL
RNase-free water	895 µL

Final concentrations: 1× buffer, 5 mM DTT.

3) Prepare RIG-I/ATP Mix (example for 100 µL):

Components	Volume
1× RIG-I Reaction Buffer	88 µL
RIG-I enzyme（1µM）	10 µL
10mM ATP	2 µL

Final concentrations: 0.1 µM RIG-I, 0.2 mM ATP.

4) Dilute Standards and Samples:

Dilute standards to 2.5, 1.25, 0.625, 0.3125, 0.156, 0.078, 0.039, 0.0195, and 0 ng/µL using 1×RIG-I Reaction Buffer.

Recommended sample concentration range: 0.01–0.2 mg/mL.

5) Mix 5µL RIG-I/ATP mix with 5µL standard/sample. Incubate at 37°C for 60 min.

6) Prepare Reagent 1 (example for 100 µL):

Components	Volume
Reagent 1-Assay buffer	79 µL

Reagent 1-Enzyme(5×)	20 μL
Reagent 1-0.5M $MnCl_2$	1 μL

Final concentrations: 1× enzyme, 5 mM $MnCl_2$.

7) Add 5 μL reaction mixture and 5 μL Reagent 1 to the 384-well plate. Gently mix for 40 to 60 seconds on a plate shaker. Incubate at room temperature for 60 min.
8) Add 10 μL Reagent 2 to each well. Gently mix for 40 to 60 seconds on a plate shaker. Incubate at room temperature for 40 min(no sealing required).
9) Measure luminescence with a microplate reader (0.25–1 s/well).Assay Type: Endpoint detection. Wavelength: Not required; full wavelength recommended if applicable.

Notes

1. Ensure the plate and reagents are fully equilibrated to room temperature before adding ADP detection reagents. Temperature gradients may cause variability between wells.
2. Use white microplates for optimal luminescence detection. For 384-well plates, use 5 μL RIG-I reaction mix, 5 μL Reagent 1, and 10 μL Reagent 2 (total 20 μL). Maintain a 1:1:2 volume ratio.
3. Avoid repeated freeze-thaw cycles. Insoluble particles in reagents do not affect performance.
4. For research use only. Not for clinical diagnosis or other non-research purposes.