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RIGI ATPase Assay

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Protocol status: Working

We use this protocol and it's working

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Abstract

Protocol for in vitro testing of RIG-I ATPase activity

Materials

For 10x Buffer:

1 M HEPES (Thermofisher, 15630080)

5 M NaCl (Thermofisher, A57006)

50 mM MgCl₂ (Sigma Aldrich, M8787)

Nuclease Free H₂O

RIG-I (Abcam, ab132502)

White 96 well plate (Millipore Sigma, MSSWNFX40)

0.6 mL PCR tube



Buffers and Protocol

1h 15m

1 Make 10x ATPase Sample Buffer:

HEPES [M] 100 millimolar (mM)

NaCl [M] 1.5 Molarity (M)

MgCl₂ [M] 10 millimolar (mM)

1.1 For making 1 mL 10x ATPase sample buffer, mix:

 100 μ L of [M] 1 Molarity (M) HEPES 300 μ L of [M] 5 Molarity (M) NaCl 200 μ L of [M] 50 millimolar (mM) MgCl₂ 400 μ L of H₂O

2 Mix components in a 0.6 mL PCR tube:

 6 μ L of nuclease Free H₂O 1 μ L of [M] 10 millimolar (mM) ATP 1 μ L of 10x ATPase Assay Buffer 1 μ L of IVT RNA [M] 1 μ g/ μ L 1 μ L of RIG-I

2.1 As controls, replace either the IVT RNA or RIG-I with H₂O.

3 Incubate the reaction at 37 °C for 01:00:00 in a thermocycler.

1h

4 Remove reaction from thermocycler and add 40 μ L of H₂O. Then transfer

 50 μ L of mixture to a well of a white 96-well plate.

5 Add 50 μ L of Cell-Titer Glo 2 mixture and incubate at Room temperature for

 00:15:00

15m



6 Measure luciferase activity within each well with 2 second gain.