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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 $MgCl_2$ (Sigma Aldrich, M8787)
Nuclease Free H_2O

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)
$MgCl_2$ [M] 10 millimolar (mM)

1.1 For making 1 mL 10x ATPase sample buffer, mix:
100 µL of [M] 1 Molarity (M) HEPES
300 µL of [M] 5 Molarity (M) NaCl
200 µL of [M] 50 millimolar (mM) $MgCl_2$
400 µL of H_2O

2 Mix components in a 0.6 mL PCR tube:
6 µL of nuclease Free H20
1 µL of [M] 10 millimolar (mM) ATP
1 µL of 10x ATPase Assay Buffer
1 µL of IVT RNA [M] 1 µg/µL
1 µL of RIG-I

2.1 As controls, replace either the IVT RNA or RIG-I with H_2O.

3 Incubate the reaction at 37 °C for 01:00:00 in a thermocycler. 1h

4 Remove reaction from thermocycler and add 40 µL of H_2O. Then transfer 50 µL of mixture to a well of a white 96-well plate.

5 Add 50 µL 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.