ROCK Activity ELISA Kit

ROCK Activity ELISA Kit is an Enzyme Immunoassay kit for the quantification of ROCK Activity in purified kinase, cell/tissue lysate.

Catalog number: ARG83613

Package: 96 wells

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PRINCIPLE OF THE ASSAY

This pair employs the quantitative sandwich enzyme immunoassay technique. Coating specific recombinant MYPT1 on a microtiter plate. Active ROCK-II or samples are pipetted into the wells and any ROCK present is bound by the immobilized antibody. After washing away any unbound substances, an anti-phosphoMYPT1 (Thr696) Detection antibody is added to each well and incubate. Following a washing to remove unbound substances, HRP-Streptavidin Solution is added to each microplate well and incubated. After washing away any unbound antibody, a substrate solution (TMB) is added to the wells and color develops in proportion to the amount of ROCK Activity bound in the initial step. The color development is stopped by the addition of acid and the intensity of the color is measured at a wavelength of 450nm.

MATERIALS PROVIDED & STORAGE INFORMATION

Upon receipt, store Standard at -80°C, ATP Solution at -20°C to avoid repeated freeze-thaw cycles, all other components store at 4°C. Use the kit before expiration date.

Component	Quantity	Storage information
recombinant MYPT1-coated microplate	12 X 8 strips	4°C
Active ROCK-II (25 mM)	20 µl	-80°C
1000X phosphor MYPT1 (Thr696) Antibody Concentrate	20 µl	4°C
1000X HRP-Streptavidin Concentrate	20 µl	4°C
Assay Diluent	50 ml	4°C
Kinase Buffer	20 ml	4°C
ATP Solution (100 mM)	100 µl	-20°C
10X Wash Buffer	100 ml	4°C
TMB substrate	12ml (Ready-to-use)	4°C (Protect from light)
STOP solution	12ml (Ready-to-use)	4°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 450nm
- Pipettes and pipette tips
- Multichannel micropipette reservoir
- Deionized or distilled water
- Microplate shaker.
- Automated microplate washer (optional)
- Lysis Buffer, DTT, 0.5 M EDTA

TECHNICAL HINTS AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Upon receipt, store Standard at -80°C, ATP Solution at -20°C to avoid repeated freeze-thaw cycles, all other components store at 4°C. Use the kit before expiration date.
- If crystals are observed in the 10X Wash buffer, warm to RT or 37°C until the crystals are completely dissolved.
- Ensure complete reconstitution and dilution of reagents prior to use.
- All reagents should be mixed by gentle inversion or swirling prior to use. Do not induce foaming.
- Before using the kit, spin tubes and bring down all components to the bottom of tubes.
- It is highly recommended that the standards and samples be assayed in duplicates.
- Change pipette tips between the addition of different reagent or samples.

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Cell or Tissue Lysate- Sonicate or homogenize sample in cold PBS and centrifuge at 10,000 x g for 10 minutes at 4°C. Collect samples and assay immediately or aliquot and store samples at -80°C. Avoid repeated freeze-thaw cycles.

REAGENT PREPARATION

- **1X Wash buffer**: Dilute 10X Wash buffer into distilled water to yield 1X Wash buffer, mix well. Storage at 2-8°C.
- **Kinase Reaction Buffer:** Dilute immediately before use; mix 1M DTT, ATP solution (100mM) and Kinase Reaction Buffer at 1:2:97 dilution (ex. add 10 µl of 1M DTT and 20 µl of mM ATP solution (100mM) into 970 µl of 10X Kinase Buffer)
- **1X phosphor MYPT1 (Thr696) Antibody:** Dilute immediately before use; dilute the **1000X phosphor MYPT1 (Thr696) Antibody Concentrate** into Assay Diluent to yield **1X phosphor MYPT1 (Thr696) Antibody**. Do not store diluted solutions.
- **1X HRP-Streptavidin:** Dilute immediately before use; dilute the **1000X HRP-Streptavidin concentrate** into Assay Diluent to yield **1X HRP-Streptavidin**. Do not store diluted solutions.
- **Active ROCK-II:** Dilute immediately before use; dilute the 10 µl Active ROCK-II stock into 25 µl Assay Diluent to yield **1X HRP-Streptavidin**. Do not store diluted solutions.

ASSAY PROCEDURE

Warm Substrate Solution to room temperature (RT) before use. Active ROCK-II, samples should be assayed in duplicates.

1. Remove excess microtiter strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal it.
2. Add **90 µl** of the **Active ROCK-II** and **samples** into the appropriate wells.
3. Add **10 µl** of the **Kinase Reaction Buffer** into each well. Incubate for **30-60 minutes** at **RT** on a microplate shaker**.**
4. Add **50 µl** of the **0.5 M EDTA** into each well, to stop the kinase reaction.
5. Aspirate each well and wash, repeating the process 2 times for a total **3 washes**. Wash by filling each well with **1× Wash Buffer (250 µl)** using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating, decanting or blotting
6. Add **100 µl** of the **1X phosphor MYPT1 (Thr696) Antibody Concentrate** to all wells and incubate for **1 hour at RT** on a microplate shaker.
7. Aspirate each well and **wash as step 5**.
8. Add **100 µl** of the **1X HRP-Streptavidin** to all wells and incubate for **1 hour at RT** on a microplate shaker.
9. Aspirate each well and **wash as step 5**.
10. Add **100 µl** of **TMB substrate solution** into each well. Incubate for **2-30 mins at RT** on microplate shaker. Avoid exposure to light.
11. Add **100 µl** of **Stop Solution** to each well. Gently tap the plate to ensure thorough mixing.
12. Read the OD with a microplate reader at **450 nm** immediately.

CALCULATION OF RESULTS

1. Calculate the average absorbance values for each set of Active ROCK-II, controls and samples.
2. Using the mean absorbance value for each sample determine the corresponding concentration from the Active ROCK-II curve.

QUALITY ASSURANCE

Sensitivity

200 pg