Ras Activation ELISA Kit

Ras Activation ELISA Kit is an Enzyme Immunoassay kit for the quantification Ras Activation cell sample.

Catalog number: ARG83384

Package: 96 wells

For research use only. Not for use in diagnostic procedures.

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INTRODUCTION

The Ras superfamily, derived from "Rat sarcoma virus", is a protein superfamily of small GTPases. Members of the superfamily are divided into families and subfamilies based on their structure, sequence and function. The five main families are Ras, Rho, Ran, Rab and Arf GTPases. The Ras family itself is further divided into 6 subfamilies: Ras, Ral, Rap, Rheb, Rad and Rit. Miro is a recent contributor to the superfamily. Each subfamily shares the common core G domain, which provides essential GTPase and nucleotide exchange activity.

The surrounding sequence helps determine the functional specificity of the small GTPase, for example the 'Insert Loop', common to the Rho subfamily, specifically contributes to binding to effector proteins such as WASP.

In general, the Ras family is responsible for cell proliferation: Rho for cell morphology, Ran for nuclear transport, and Rab and Arf for vesicle transport.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. An antibody specific for RAS has been pre-coated onto a microtiter plate. Samples are pipetted into the wells and any RAS present is bound by the immobilized antibody. After washing away any unbound substances, a biotin-conjugated antibody specific for RAS is added to each well and incubate. Following a washing to remove unbound substances, streptavidin conjugated to Horseradish Peroxidase (HRP) is added to each microplate well and incubated. After washing away any unbound antibody-enzyme reagent, a substrate solution (TMB) is added to the wells and color develops in proportion to the amount of RAS 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 ± 2nm.

MATERIALS PROVIDED & STORAGE INFORMATION

Upon received, store Raf-1 RBD at ≤ -80°C, store 1000X Ras Activation Antibody and control at ≤ -20°C, store other component at 2-8°C at all times.

Component	Quantity	Storage information
Antibody Coated Microplate	8 x 12 strips	4°C.
Raf-1 RBD	40 µL	≤ -80°C
1000X pan-Ras Antibody	1 vial (20 µL)	≤ -20°C
1000X HRP-Streptavidin Solution	1 vial (20 µL)	4°C
Diluent Buffer	50 mL	4°C
10X Wash Buffer	100 mL	4°C
5X Lysis Buffer	30 mL	4°C
TMB Substrate	12 mL	4°C (Protect from light)
Control	2 X 50 µL	≤ -20°C
Stop Solution	12 mL	4°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of reading at O.D. 450 nm
- Centrifuge and centrifuge tube
- Deionized or Distilled water
- Pipettes, pipette tips and Multichannel micropipette reservoir
- Automated microplate washer (optional)

TECHNICAL NOTES AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Upon received, store Raf-1 RBD at ≤ -80°C, store 1000X Ras Activation Antibody and control at ≤ -20°C, store other component at 2-8°C at all times. Use the kit before expiration date.
- Prior to beginning the assay procedure, bring all reagents and required number of strips to room temperature.
- Briefly spin down the all vials before use.
- If crystals are observed in the 10X Wash Buffer, warm to 37°C until the crystals are completely dissolved.
- Minimize lag time between wash steps to ensure the plate does not become completely dry during the assay.
- Ensure complete reconstitution and dilution of reagents prior to use.
- Take care not to contaminate the TMB Substrate. Do not expose the TMB solution to glass, foil or metal. Do NOT return leftover TMB Substrate to bottle. Do NOT contaminate the unused TMB Substrate. If the solution is blue before use, DO NOT USE IT.
- Change pipette tips between the addition of different reagent or samples.
- Taping the well strips together with lab tape can be done as an extra precaution to avoid plate strips from coming loose during the procedure.

REAGENT PREPARATION

- **1X Wash Buffer:** Dilute 10X Wash Buffer into distilled water to yield **1X** Wash Buffer.
- **1X Lysis Buffer**: Dilute 5X Lysis Buffer into distilled water to yield **1X** Lysis Buffer. Add protease inhibitors just before use.
- **1X pan-Ras Antibody:** 10 minutes before use, dilute **1000X** pan-Ras Antibody into Diluent Buffer to yield **1X** pan-Ras Antibody.
- **1X HRP-Streptavidin Solution:** 10 minutes before use, dilute **2500X** HRP-Streptavidin Solution into Diluent Buffer to yield **1X** HRP-Streptavidin Solution. Keep diluted HRP-Streptavidin Solution in dark before use.

SAMPLE COLLECTION & STORAGE INFORMATION

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

Suspension Cells: Centrifuge 3-6 x 10^6 cells at 1200 x g for 2 minutes and discard supernatant. Wash cell pellet twice with ice-cold PBS, centrifuge, and discard the supernatant. Resuspend cell pellet in 0.5 - 1 mL of cold 1X Lysis Buffer. Lyse cells with repeated pipetting on ice. Centrifuge at 12000 x g for 10 minutes and collect the cell lysate supernatant.

Adherent Cells: Wash 1-5 x 10^6 cells twice with ice-cold PBS per 100 mm dish, and add 0.5 - 1 mL of ice-cold 1X Lysis Buffer for 10 – 20 min. Harvest cells with a cell scraper. Centrifuge at 14000 x g for 10 minutes at 4°C and collect the cell lysate supernatant.

ASSAY PROCEDURE

All materials should be equilibrated to room temperature (RT, 20-25°C) before use. All assay should be assayed in duplicates.

Control Loading

1. Add 0.5 mL same samples to two microcentrifuge tubes.
2. Add 10 µL 0.5 M EDTA to each sample.
3. Add 5 µL of positive control and 5 µL of 100X negative control to the other tube.
4. Mix and incubate for 30 minutes at 30°C.
5. Add 33 µL of 1 M MgCl2 to each tube for stopping the loading.
6. Mix and place tubes on ice.

Ras Activation ELISA step

1. Determine the number of wells to be used, and dilute the Raf-1 RBD 1:500 in Assay Diluent. Add **100 µL** of **1X** Raf-1 RBD into each wells the plate.
2. Cover the plate and incubate for **1 hour** at **RT**.
3. Aspirate each well and wash, repeating the process 2 time for a **total 3 washes.** Wash by filling each well with **1X 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 against clean paper towels.
4. Add **50 µL** of **lysate sample**, **control** and **blank** to well.
5. Add **50 µL** of **Diluent Buffer** to each well (100 µL total volume).
6. Cover the plate and incubate for **1 hour** at **RT**.

7. Aspirate each well and **wash** plate **as step 3**, but for5 5 times wash.
8. Add **100 µL** of **1X pan-Ras Antibody** to each well.
9. Cover the plate and incubate for **1 hour** at **RT**.
10. Aspirate each well and **wash** plate **as step 7**.
11. Add **100 µL** of **1X HRP-Streptavidin Solution** to each well.
12. Cover the plate and incubate for **1 hour** at **room temperature** in the dark.
13. Aspirate each well and **wash** plate **as step 7**.
14. Add **100 µL** of **TMB Substrate** in each well.
15. Incubate for **5-20 mins** at **room temperature** in the dark.
16. Add **100 µL** of **Stop Solution** to each well to stop the reaction.
17. Read the absorbance with a plate reader at **O.D. 450 nm.** It is recommended reading the absorbance within 10 minutes after adding the stop solution.