Datasheet for ABIN2345103

RAS ELISA Kit

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Overview

Quantity:	96 tests
Target:	RAS
Reactivity:	Various Species
Method Type:	Sandwich ELISA
Application:	ELISA

Product Details

Sample Type:	Cell Samples, Tissue Lysate
Analytical Method:	Quantitative
Detection Method:	Colorimetric
Characteristics:	96-well Ras Activation ELISA Kit utilizes plate-bound, Raf-1 RBD to selectively isolate and pull-down the active forms of Ras (H-, K-, and N-Ras isoforms from human, mouse and rat) from purified samples or endogenous lysates. Subsequently, the captured GTP-Ras is detected by an Anti- pan-Ras Antibody and HRP conjugated secondary antibody. 96-well Ras Activation ELISA Kit provides a simple and fast tool to monitor the activation of Ras. Each kit provides sufficient reagents to perform up to 96 assays.
Components:	1. Raf-1 RBD Capture Plate : One 96-well strip plate (8 x 12). 2. 5X Assay/Lysis Buffer : One 30 mL bottle of 125 mM HEPES, pH 7.5, 750 mM NaCl, 5% NP-40, 50 mM MgCl2, 5 mM EDTA, 10% Glycerol. 3. Assay Diluent : One 50 mL bottle. 4. 10X Wash Buffer : One 100 mL bottle. 5. Substrate Solution : One 12 mL amber bottle. 6. Stop Solution (Part. No. 310808): One 12 mL bottle. 2

Product Details

	Box 2 (shipped on blue ice packs)
Material not included:	1. Stimulated and non-stimulated cell or tissue lysates 2. Ras activators 3. Protease inhibitors 4. 0.5 M EDTA in water 5. 1 M MgCl2 6. 30 °C incubator or water bath 7. Room temperature shaker 8. 10 µL to 1000 µL adjustable single channel micropipettes with disposable tips 9. 50 µL to 300 µL adjustable multichannel micropipette with disposable tips 10. Multichannel micropipette reservoir 11. Microplate reader capable of reading at 450 nm (620 nm as optional reference wave length)

Target Details

Target:	RAS
Abstract:	RAS Products
Background:	Small GTP-binding proteins (or GTPases) are a family of proteins that serve as molecular regulators in signaling transduction pathways. Ras, a 21 kDa protein, regulates a variety of biological response pathways that include cell growth, cell transformation and tumor invasion. Like other small GTPases, Ras regulates molecular events by cycling between an inactive GDP-bound form and an active GTP- bound form. In its active (GTP-bound) state, Ras binds specifically to the Ras-binding domain (RBD) of Raf-1 to control downstream signaling cascades. The most notable members of the Ras subfamily are H-Ras, N-Ras and K-Ras, mainly for being implicated in many types of cancer.

Application Details

Application Notes:	Optimal working dilution should be determined by the investigator.
Comment:	• Raf1 RBD detects only the active form of Ras • Convenient 96-well strip-well format
Plate:	Pre-coated
Reagent Preparation:	• 1X Assay/Lysis Buffer: Mix the 5X Assay/Lysis Buffer briefly and dilute to 1X in deionized water. Just prior to usage, add protease inhibitors such as 1 mM PMSF, 10 µg/mL leupeptin, and 10 µg/mL aprotinin. • 1X Wash Buffer: Dilute the 10X Wash Buffer to 1X with deionized water. Stir to homogeneity. • Anti-pan-Ras Antibody: Immediately before use dilute the Anti-pan-Ras Antibody 1:1000 with

	Assay Diluent. Do not store diluted solutions. • Secondary Antibody, HRP Conjugate: Immediately before use dilute the Secondary Antibody, HRP Conjugate 1:2500 with Assay Diluent. Do not store diluted solutions. 3
Sample Preparation:	Note: It is advisable to use fresh cell or tissue lysates because GTP-Ras is quickly hydrolyzed to GDP-Ras, frozen lysates stored at -70 °C may be used. Performing steps at 4 °C or on ice may reduce hydrolysis. Avoid multiple freeze/thaw cycles of lysates. I. Adherent Cells 1. Culture cells to approximately 80-90 % confluence. Stimulate cells with Ras activator(s) as desired. 2. Aspirate the culture media and wash twice with ice-cold PBS. 3. Completely remove the final PBS wash and add ice-cold 1X Assay/Lysis Buffer to the cells (0.5 - 1 mL per 100 mm tissue culture plate). 4. Place the culture plates on ice for 10-20 minutes. 5. Detach the cells from the plates by scraping with a cell scraper. 6. Transfer the lysates to appropriate size tubes and place on ice. 7. If nuclear lysis occurs, the cell lysates may become very viscous and difficult to pipette. If this occurs, lysates can be passed through a 271/2-gauge syringe needle 3-4 times to shear the genomic DNA. 8. Clear the lysates by centrifugation for 10 minutes (14,000 x g at 4 °C). 9. Collect the supernatant and store samples on ice for immediate use, or snap freeze samples and store at -70 °C for future use. 10. Proceed to GTPγS/GDP Loading for positive and negative controls, or the Activation ELISA (Assay Protocol Section). II. Suspension Cells 1. Culture cells and stimulate with Ras activator(s) as desired. 2. Perform a cell count, and then pellet the cells by centrifugation. 3. Aspirate the culture media and wash twice with ice-cold PBS. 4. Completely remove the final PBS wash and add ice-cold 1X Assay/Lysis Buffer to the cell pellet (0.5 - 1 mL per 1 x 107 cells). 5. Lyse the cells by repeated pipetting. 6. Transfer the lysates to appropriate size tubes and place on ice. 7. If nuclear lysis occurs, the cell lysates may become very viscous and difficult to pipette. If this occurs, lysates can be passed through a 271/2-gauge syringe needle 3-4 times to shear the genomic DNA. 8. Clear the lysates by centrifugation for 10 minutes (14,000 x g at 4 °C). 9. Collect the supernatant and store samples on ice for immediate use, or snap freeze samples and store at -70 °C for future use. 4 10. Proceed to GTPγS/GDP Loading for positive and negative controls, or the Activation ELISA (Assay Protocol Section).
Assay Procedure:	I. GTPγS/GDP Loading (Positive and Negative Controls) Note: Samples that will not be GTPγ

S/GDP loaded may be kept on ice during preparation of GTPγS/GDP loading samples.

1. Aliquot 0.5 mL of each cell lysate to two microcentrifuge tubes. Note: Typical protein concentration of sample is > 0.5 mg/mL.
2. Add 10 µL of 0.5 M EDTA to each sample.
3. Add 5 µL of 100X GTPγS to one tube (positive control) and 5 µL of 100X GDP to the other tube (negative control). Mix and label each tube appropriately.
4. Incubate the tubes for 30 minutes at 30 °C with agitation.
5. Stop the loading by adding 33 µL of 1 M MgCl2 to each tube. Mix and place tubes on ice.
6. Continue with the Activation ELISA.

II. Ras Activation ELISA Note: Samples and controls should be thawed/maintained on ice just prior to use (Step 3).

1. Determine the number of wells to be used, and dilute the Raf-1 RBD 1:500 in Assay Diluent. Add 100 µL of the diluted Raf-1 RBD to each well of the Raf-1 RBD Capture Plate. Incubate at room temperature for 1 hour on an orbital shaker. Note: Do not store diluted solutions.
2. Wash microwell strips 3 times with 250 µL 1X Wash Buffer per well with thorough aspiration between each wash. After the last wash, empty wells and tap microwell strips on absorbent pad or paper towel to remove excess 1X Wash Buffer.
3. Add 50 µL of Ras lysate sample (10-100 µg), control, or buffer blank per well. Each sample should be assayed in duplicate. Any sample dilutions should be performed in cold, 1X Assay/Lysis Buffer.
4. Immediately add 50 µL of Assay Diluent to each well (100 µL total volume). Incubate at room temperature for 1 hour on an orbital shaker.
5. Wash microwell strips 5 times with 250 µL 1X Wash Buffer per well with thorough aspiration between each wash. After the last wash, empty wells and tap microwell strips on absorbent pad or paper towel to remove excess 1X Wash Buffer. 5
6. Add 100 µL of the diluted Anti-pan-Ras Antibody to each well. Incubate at room temperature for 1 hour on an orbital shaker.
7. Wash the strip wells 5 times according to step 5 above.
8. Add 100 µL of the diluted Secondary Antibody, HRP Conjugate to each well. Incubate at room temperature for 1 hour on an orbital shaker.
9. Wash the strip wells 5 times according to step 5 above. Proceed immediately to the next step.
10. Warm Substrate Solution to room temperature. Add 100 µL of Substrate Solution to each well, including the blank wells. Incubate at room temperature on an orbital shaker. Actual incubation time may vary from 5-20 minutes. Note: Watch plate carefully, if color changes rapidly, the reaction may need to be stopped sooner to prevent saturation.
11. Stop the enzyme reaction by adding 100 µL of Stop Solution into each well, including the blank wells. Results should be read immediately (color will fade over time).
12. Read absorbance of each microwell on a spectrophotometer using 450 nm as the primary wave length. 6 Example of Results The following figure demonstrates typical results seen with Cell Biolabs 96-well Ras Activation ELISA Kit. One should use the data below for reference only. Figure 1: EGF Stimulation. HeLa cells were serum starved for 18 hours before

Application Details

	EGF stimulation (50 ng/mL for 2 minutes). Lysates were then prepared according to Assay Protocol. Background has been subtracted from data. Figure 2: Pan-Ras Antibody Specificity. Anti-pan-Ras Antibody specificity to purified, H-, K-, and N-Ras human isoforms by dot blot.
Restrictions:	For Research Use only

Handling

Storage:	-20 °C/-80 °C
Storage Comment:	Upon receipt, aliquot and store Raf-1 RBD at -80°C and avoid freeze/thaw. Aliquot and store the anti- pan-Ras Antibody, GTPγS, and GDP components at -20°C and avoid freeze/thaw. Store all other components at 4°C.