ab324998 – Hamster (CHO) GTP-Binding Nuclear Protein (RAN)

ELISA Kit

For the quantitative measurement of

GTP-Binding Nuclear Protein (RAN) in Hamster Tissue Culture Fluids.

For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit:
http://www.abcam.com/ab324998

Storage and Stability:

The entire kit may be stored at 2-8°C for up to 1 year from the date of shipment.

Materials Supplied

Item	Quantity	Storage Condition
ELISA Micro Plate, antibody coated	1 unit	+4°C
Detection Antibody 100X	150 µL	+4°C
HRP-Streptavidin 100X	150 µL	+4°C
Calibrator	1 vial	+4°C
Diluent Concentrate	50 mL	+4°C
Wash Solution Concentrate	50 mL	+4°C
Chromogen Substrate Solution	12 mL	+4°C – Store in the dark
STOP Solution	12 mL	+4°C

Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully utilize this assay:

- Precision pipettes (2 µL to 100 µL) for making and dispensing dilutions.
- Test tubes.
- Squirt bottle or Microtitre washer/aspirator.
- Distilled or Deionized H_2O.
- Microtitre Plate reader.
- Assorted glassware for the preparation of reagents and buffer solutions.
- Centrifuge for sample collection.
- Anticoagulant for plasma collection.
- Timer.
- Microplate shaker.

Specimen Collection and Handling

Known interfering substances: Azide and thimerosal at concentrations higher than 0.1% inhibits the enzyme reaction.

Dilution of Samples

The assay requires that each test sample be diluted before use. All samples should be assayed in duplicate each time the assay is performed. The recommended dilutions are only suggestions. Dilutions should be based on the expected concentration of the unknown sample such that the diluted sample falls within the dynamic range of the standard curve. If unsure of sample level, a serial dilution with one or two representative samples before running the entire plate is highly recommended.

Supernatant Samples: Recommended starting dilution is 1/20. To prepare a 1/20 dilution of a sample, transfer 30 µL of sample to 570 µL of 1X diluent. This gives you a 1/20 dilution. Mix thoroughly.

Reagent Preparation

- Equilibrate all reagents to room temperature (18-25°C) prior to use. The kit contains enough reagents for 96 wells.
- Prepare only as much reagent as is needed on the day of the experiment.

Diluent Concentrate: The Diluent Solution supplied is a 5X Concentrate and must be diluted 1/5 with distilled or deionized water (1 part buffer concentrate, 4 parts dH_2O).

Wash Solution Concentrate: The Wash Solution supplied is a 20X Concentrate and must be diluted 1/20 with distilled or deionized water (1 part buffer concentrate, 19 parts dH_2O). Crystal formation in the concentrate may occur when storage temperatures are low. Warming of the concentrate to 30-35°C before dilution can dissolve crystals.

Detection Antibody: Calculate the required amount of working conjugate solution for each microtitre plate test strip by adding 10 µL Detection Antibody to 990 µL of 1X Diluent for each test strip to be used for testing. Dilute immediately before use and protect from light. Mix uniformly, but gently. Avoid foaming.

HRP-Streptavidin: Calculate the required amount of working conjugate solution for each microtitre plate test strip by adding 10 µL HRP-Streptavidin to 990 µL of 1X Diluent for each test strip to be used for testing. Dilute immediately before use and protect from light. Mix uniformly, but gently. Avoid foaming.

Pre-coated ELISA Micro Plate: Ready to use as supplied. Unseal foil pouch and remove plate from pouch. Remove all strips and wells that will not be used in the assay and place back in pouch and re-seal along with desiccant.

Calibrator: Prepare according to the lot specific Certificate of Analysis.

Assay Procedure

Equilibrate all materials and prepared reagents to room temperature prior to use. We recommend that you assay all standards, controls and samples in duplicate. Prepare all reagents, working standards, and samples as directed in the previous sections.

1. The Standards and the test sample(s) should be loaded into the ELISA wells as quickly as possible to avoid a shift in OD readings. Using a multichannel pipette would reduce this occurrence.

   Pipette 100 µL of:

   - Standard 0: (0.0 ng/mL) in duplicate
   - Standard 1: (0.31 ng/mL) in duplicate
   - Standard 2: (0.63 ng/mL) in duplicate
   - Standard 3: (1.25 ng/mL) in duplicate
   - Standard 4: (2.5 ng/mL) in duplicate
   - Standard 5: (5 ng/mL) in duplicate
   - Standard 6: (10 ng/mL) in duplicate
   - Standard 7: (20 ng/mL) in duplicate

2. Pipette 100 µL of sample (in duplicate) into pre designated wells.

3. Incubate the micro titer plate while shaking on a microplate shaker at 400 rpm at room temperature for one hundred and twenty (120 ± 2) minutes. Keep plate covered and level during incubation.

4. Following incubation, aspirate the contents of the wells.

5. Completely fill each well with appropriately diluted Wash Solution and aspirate. Repeat three times, for a total of four washes. If washing manually: completely fill wells with wash buffer, invert the plate then pour/shake out the contents in a waste container. Follow this by sharply striking the wells on absorbent paper to remove residual buffer. Repeat 3 times for a total of four washes.

6. Pipette 100 µL of appropriately diluted Detection Antibody to each well. Incubate while shaking on a microplate shaker at 400 rpm at room temperature for twenty (20 ± 2) minutes. Keep plate covered in the dark and level during incubation.

7. Wash and blot the wells as described in Steps 4/5.

8. Pipette 100 µL of appropriately diluted HRP-streptavidin to each well. Incubate while shaking on a microplate shaker at 400 rpm at room temperature for twenty (20 ± 2) minutes. Keep plate covered in the dark and level during incubation.

9. Wash and blot the wells as described in Steps 4/5.

10. Pipette 100 µL of TMB Substrate Solution into each well.

11. Incubate in the dark while shaking on a microplate shaker at 400rpm at room temperature for precisely ten (10) minutes. Keep plate covered in the dark and level during incubation.

12. After ten minutes, add 100 µL of Stop Solution to each well.

13. Determine the absorbance (450 nm) of the contents of each well within 30 minutes. Calibrate the plate reader to manufacturer's specifications.

Calculation of results

Subtract the average background value (Average absorbance reading of Standard zero) from the test values for each sample.

Average the duplicate readings for each standard and use the results to construct a Standard Curve. Construct the standard curve by reducing the data using computer software capable of generating a four parameter logistic curve fit. A second order polynomial (quadratic) or other curve fits may also be used; however, they will be a less precise fit of the data.

Interpolate test sample values from standard curve. Correct for sera dilution factor to arrive at the RAN concentration in original samples.

Download our ELISA guide for technical hints, results, calculation, and troubleshooting tips:
www.abcam.com/protocols/the-complete-elisa-guide

For technical support contact information, visit: www.abcam.com/contactus

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