Version 2b Last updated 12 March 2026

ab212011
Rap1 Activation Assay Kit

For the rapid, sensitive and accurate measurement of active Rap1 in cell or tissue lysates from human, mouse or rat.

This product is for research use only and is not intended for diagnostic use.

Table of Contents

1. Overview

Rap1 Activation Assay Kit (ab212011) uses RalGDS RBD Agarose beads to selectively isolate and pull-down the active form of Rap from purified samples or endogenous lysates. Subsequently, the precipitated GTP-Rap is detected by western blot analysis using an anti-Rap1 polyclonal antibody, which reacts with the human, mouse and rat Rap1A and Rap1B proteins.

Features:

- Non radioactive assay format
- Fast results: 1-hour assay plus electrophoresis/blotting time
- Includes Rap1 positive control
- Pink colored agarose beads for easy identification during washing and aspiration steps

Small GTP-binding proteins (or GTPases) are a family of proteins that serve as molecular regulators in signaling transduction pathways.

Rap1 (Ras-related protein 1), a 21 kDa protein of the Ras superfamily, exhibits 60% identity to Rap2. Rap2 regulates a variety of biological response pathways that include cell migration, cell adhesion, and embryonic blood vessel formation. Like other small GTPases, Rap1 regulates molecular events by cycling between an inactive GDP-bound form and an active GTP-bound form. In its active (GTP-bound) state, Rap1 bind specifically to the Rap-binding domain (RBD) of RalGDS to control downstream signaling cascades.

2. Protocol Summary

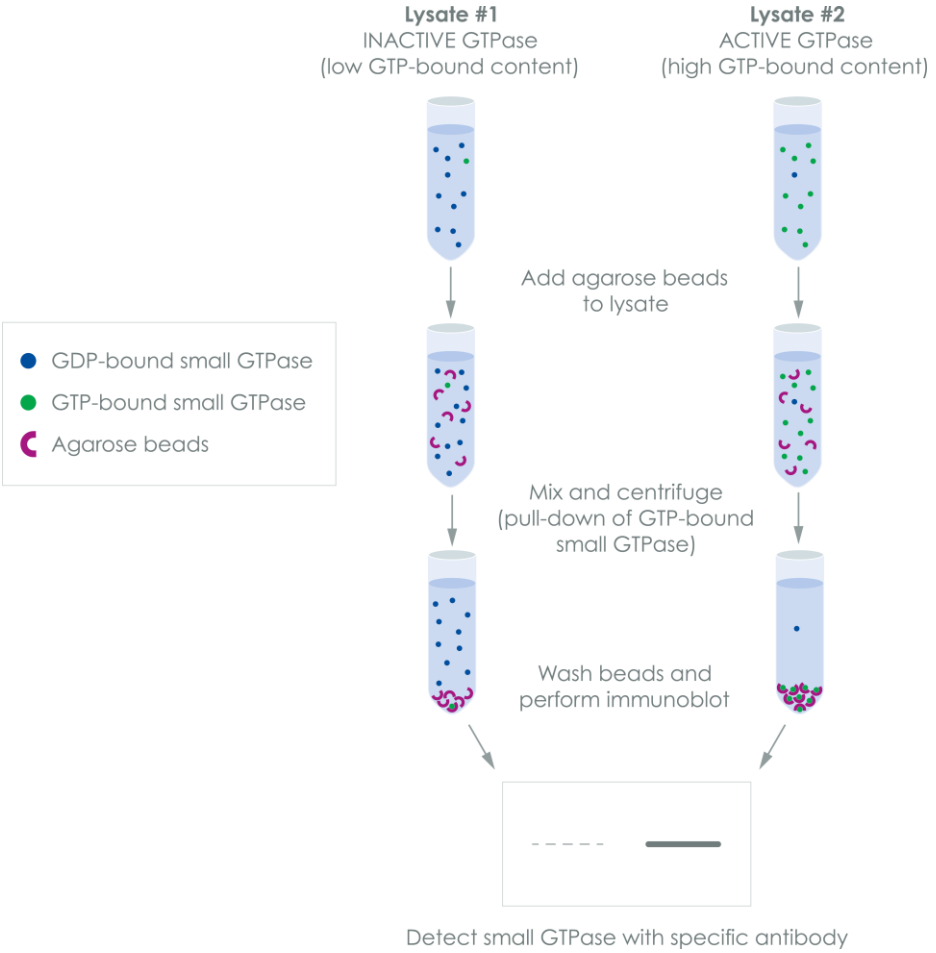

Figure 1. Small GTPase Activation Assay Principle.

3. Precautions

Please read these instructions carefully prior to beginning the assay.

- All kit components have been formulated and quality control tested to function successfully as a kit.
- We understand that, occasionally, experimental protocols might need to be modified to meet unique experimental circumstances. However, we cannot guarantee the performance of the product outside the conditions detailed in this protocol booklet.
- Observe good laboratory practices. Gloves, lab coat, and protective eyewear should always be worn. Never pipet by mouth. Do not eat, drink or smoke in the laboratory areas.
- If applicable, please refer to the current Safety Data Sheet (SDS) provided with this product for safety, handling, and disposal information. The most up to date and current versions are available on our website https://www.abcam.com/en-us.

4. Storage and Stability

Store kit at -20°C in the dark immediately upon receipt. Kit has a storage time of 1 year from receipt, providing components have not been reconstituted.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in the Materials Supplied section.

Aliquot components in working volumes before storing at the recommended temperature.

∆ Note: Avoid multiple freeze/thaw cycles.

5. Limitations

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

6. Materials Supplied

Item	Quantity	Storage condition (before prep)	Storage condition (after prep)
RalGDS RBD agarose beads (50% slurry in PBS/50% glycerol)	800 µL	-20°C	-20°C
100X GTPγS (10 mM)	50 µL	-20°C	-20°C
100X GDP (100 mM)	50 µL	-20°C	-20°C
5X Assay/Lysis Buffer	30 mL	-20°C	-20°C/4°C
Anti Rap1 Goat polyclonal antibody	40 µL	-20°C	-20°C
Rap1 immunoblot positive control	100 µL	-20°C	-20°C

7. Materials Required, Not Supplied

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

- MilliQ water or other type of double distilled water (ddH_2O)
- PBS
- Dounce homogenizer (if using tissue)
- Protease inhibitors: we recommend Protease Inhibitor Cocktail II (ab201116) [AEBSF, aprotinin, E-64, EDTA, leupeptin] as general use cocktail.
- 0.5M EDTA in ddH_2O
- 1 M $MgCl_2$
- 2X reducing SDS-PAGE sample buffer
- Polyacrylamide gel for protein separation
- Standard instruments for SDS-PAGE and western blot analysis immunoblotting systems
- Immunoblotting wash buffer such as TBST (10 mM Tris-HCl, pH 7.4, 0.15 M NaCl, 0.05% Tween-20)
- Immunoblotting blocking buffer (TBST + 5% non-fat dry milk)
- PVDF or nitrocellulose membrane
- ECL Detection Reagents: we recommend Optiblot ECL Detect Kit (ab133406)
- Secondary antibody: we recommend Donkey anti-Goat IgG H&L (HRP) (ab205723)
- Pipettes and pipette tips, including multi-channel pipette
- Assorted glassware for the preparation of reagents and buffer solutions
- Tubes for the preparation of reagents and buffer solutions
- Tube rocker or shaker (at 4°C)

8. Technical Hints

- This kit is sold based on number of tests. A "test" simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.
- Selected components in this kit are supplied in surplus amount to account for additional dilutions, evaporation, or instrumentation settings where higher volumes are required. They should be disposed of in accordance with established safety procedures.
- Avoid foaming or bubbles when mixing or reconstituting components.
- Avoid cross contamination of samples or reagents by changing tips between sample and reagent additions.
- Ensure all reagents and solutions are at the appropriate temperature before starting the assay.
- Make sure all necessary equipment is switched on and set at the appropriate temperature.

9. Reagent Preparation

Briefly centrifuge small vials at low speed prior to opening.

9.1 RalGDS RBD agarose beads:

Ready to use as supplied. Resuspend thoroughly prior use by pipetting up and down. We recommend cutting the end of the pipet tip to ensure a homogenous slurry mix. Keep on ice while in use. Store at -20°C.

9.2 100X GTPγS (10 mM):

Ready to use as supplied. Keep on ice while in use. Aliquot 100X GTPγS so that you have enough to perform the desired number of assays. Store at -20°C.

9.3 100X GDP (100 mM):

Ready to use as supplied. Keep on ice while in use. Aliquot 100X GDP so that you have enough to perform the desired number of assays. Store at -20°C.

9.4 5X Assay/Lysis Buffer:

Mix the 5X stock briefly and dilute to 1X with ddH_2O. Label reagent as **1X Assay Buffer**. Keep on ice while in use. Store diluted buffer at 4°C or at -20°C.

9.5 Anti Rap1 goat polyclonal antibody:

Ready to use as supplied. Keep on ice while in use. Aliquot antibody so that you have enough to perform the desired number of assays. Store at -20°C.

9.6 GTPase immunoblot positive control (NIH3T3 cell lysate at 0.75 mg/mL):

Ready to use as supplied. Aliquot positive control so that you have enough to perform the desired number of assays. Store at - 20°C.

10. Sample Preparation

General sample information:

- We recommend that you use fresh samples. If you cannot perform the assay at the same time, we suggest that you complete the Sample Preparation step before snap freezing your samples in liquid nitrogen and storing them immediately at -80°C. This is especially necessary if you have many samples. Do not store lysates for longer than 30 days.
- Add protease inhibitors to 1X Assay Buffer immediately prior use.
- GTP-Rap1 is very labile and is quickly hydrolyzed to GDP-Rap1. Work quickly and perform steps at 4°C or on ice to reduce hydrolysis.

10.1 Adherent Cells:

10.1.1 Culture cells to approximately 80 – 90% confluence. Stimulate cells with Rap1 activator(s) as required.

10.1.2 Aspirate culture media and wash cells twice with ice-cold PBS.

10.1.3 Resuspend cells in 1X Assay Buffer (0.5 – 1 mL/100 mm culture plate).

Δ Note: Adjust the amount of 1X Assay Buffer depending on your cell type and size of culture plate used.

10.1.4 Place culture plates on ice 10 – 20 minutes.

10.1.5 Detach cells from the plates by scraping with a cell scraper.

10.1.6 Transfer lysates to appropriate size tubes and place on ice.

Δ Note: If nuclear lysis occurs, cell lysates may become viscous and difficult to pipette. If this occurs, lysates can be passed through a 27½ -gauge syringe needed 3 – 4 times to shear the genomic DNA.

10.1.7 Centrifuge 10 minutes at 4°C at 14,000 x g in a cold centrifuge to remove any insoluble material.

10.1.8 Collect supernatant and transfer to a new tube.

10.1.9 Keep on ice.

10.2 Suspension Cells:

10.2.1 Culture cells and stimulate cells with Rap1 activator(s) as required.

10.2.2 Count cells and harvest amount of cells necessary for each assay (initial recommendation = 1 x 10^7 cells).

10.2.3 Aspirate culture media and wash cells twice with ice-cold PBS.

10.2.4 Resuspend cells in 1X Assay Buffer (0.5 – 1 mL/1 x 10^7 cells).

Δ Note: Adjust the amount of 1X Assay Buffer depending on your cell type and size of culture plate used.

10.2.5 Homogenize cells quickly by pipetting up and down a few times.

10.2.6 Transfer lysates to appropriate size tubes and place on ice.

Δ Note: If nuclear lysis occurs, cell lysates may become viscous and difficult to pipette. If this occurs, lysates can be passed through a 27½ -gauge syringe needed 3 – 4 times to shear the genomic DNA.

10.2.7 Centrifuge 10 minutes at 4°C at 14,000 x g in a cold centrifuge to remove any insoluble material.

10.2.8 Collect supernatant and transfer to a new tube.

10.2.9 Keep on ice.

10.3 Tissue Lysates:

10.3.1 Harvest the amount of tissue necessary for each assay (initial recommendation = 20 - 50 mg).

10.3.2 Wash tissue in ice-cold PBS.

10.3.3 Resuspend tissue in 1X Assay Buffer (1 mL/20 – 50 mg tissue).

10.3.4 Homogenize tissue with a Dounce homogenizer or pestle sitting on ice, with 10 – 15 passes.

10.3.5 Homogenize tissue with a Dounce homogenizer or pestle sitting on ice, with 10 – 15 passes.

10.3.6 Centrifuge 10 minutes at 4°C at 14,000 x g in a cold centrifuge to remove any insoluble material.

10.3.7 Collect supernatant and transfer to a new tube.

10.3.8 Keep on ice.

11. Assay Procedure

- Equilibrate all materials and prepared reagents to room temperature prior to use.
- Prepare all reagents and samples as directed in the previous sections.

Δ Note: Using sufficient lysate in the pull-down assay is critical for success. Before running any small GTPase pull-down assay, we recommend running a WB directly on the cell/tissue lysate using the antibody provided in the kit. For example: load 5 µg – 10 µg – 20 µg of lysates onto an SDS-PAGE gel, transfer and blot. When performing the pull-down assay, use 100-times the amount of lysate that gave you a clear band of your desired small GTPase in the direct WB. For example: if the 5 µg band was faint but the 10 µg was clear and strong, use 100 x 10 µg = 1 mg of lysate in the assay.

11.1 Positive and Negative Control Preparation (GTPγS/GDP loading):

11.1.1 Aliquot 0.5 – 1 mL of each different cell lysate to two microcentrifuge tubes. Typical protein content/sample is > 0.5 mg.

Δ Note: If nuclear lysis occurs, cell lysates may become viscous and difficult to pipette. If this occurs, lysates can be passed through a 27½ -gauge syringe needed 3 – 4 times to shear the genomic DNA.

11.1.2 Adjust each sample volume up to 1 mL with 1X Assay Buffer.

11.1.3 Add 20 µL of 0.5 M EDTA to each sample.

11.1.4 Positive control: add 10 µL GTPγS (Step 9.2) to the tube. Mix and label.

11.1.5 Negative control: add 10 µL GDP (Step 9.3) to the tube. Mix and label.

11.1.6 Incubate the tubes for 30 minutes at 30°C with agitation.

11.1.7 Stop the loading by adding 65 µL of 1 M $MgCl_2$ to each tube. Mix and place tubes on ice.

11.2 Rap1 Pull-Down Assay:

11.2.1 Aliquot 0.5 – 1 mL of cell lysate (untreated or treated with Rap1 activators) to a microcentrifuge tube. Keep tubes on ice.

11.2.2 Adjust each sample volume up to 1 mL with 1X Assay Buffer.

11.2.3 Thoroughly resuspend the RalGDS RBD agarose bead slurry by vortexing or triturating.

11.2.4 Quickly add 40 µL of resuspended RalGDS RBD bead slurry (Step 9.1) to each tube of sample, positive and negative control.

11.2.5 Incubate tubes at 4°C for 1 hour with gentle agitation.

11.2.6 Pellet the beads by centrifugation for 10 seconds at 14,000 x g.

11.2.7 Aspirate supernatant, making sure not to disturb/remove the bead pellet.

Δ Note: We recommend reserving and keeping supernatant in another tube until experiment is finished. Load 20 µL of supernatant onto the SDS-PAGE gel as a control for the pull-down.

11.2.8 Wash the bead pellet 3 times with 0.5 mL of 1X Assay Buffer, centrifuging and aspirating each time.

11.2.9 After the last wash, pellet the beads and carefully remove all the supernatant.

11.2.10 Resuspend the bead pellet in 40 µL of 2X reducing SDS-PAGE sample buffer.

11.2.11 Boil each sample for 5 minutes.

11.2.12 Centrifuge each sample for 10 seconds at 14,000 x g.

11.3 Electrophoresis and Transfer:

11.3.1 Load 20 µL/well of pull-down supernatant to a 12 – 17% polyacrylamide gel. Include a pre-stained MW standard to use as indicator for successful transfer.

Δ Note: Add 10 µL/well of GTPase immunoblot positive control (Step 9.6) to gel as an immunoblot positive control.

11.3.2 Perform SDS-PAGE as per manufacturer's instructions.

11.3.3 Transfer the gel protein to a PVDF or nitrocellulose membrane as per manufacturer's instructions.

Δ Note: PVDF membrane needs to be activated by immersing PVDF membrane in 100% methanol for 15 seconds prior soaking in transfer buffer.

11.4 Immunoblotting and Detection:

11.4.1 Following transfer step, block the membrane with 5% non-fat dry milk in TBST for 1 hour at RT with constant agitation.

11.4.2 Dilute goat anti-Rap1 antibody (Step 9.5) at 1:200 to 1:1000 dilution in 5% non-fat dry milk/TBST. Incubate membrane with primary antibody for 1 – 2 hour at RT with constant agitation.

11.4.3 Wash membrane three times with TBST, 5 minutes each time, at RT with constant agitation.

11.4.4 Incubate the membrane with the appropriate secondary antibody, freshly diluted in 5% non-fat dry milk/TBST, for 1 hour at RT with constant agitation.

11.4.5 Wash membrane three times with TBST, 5 minutes each time, at RT with constant agitation.

11.4.6 Detect signal with the detection method of your choice.

12. Typical Data

Typical data – data provided **for demonstration purposes only**.

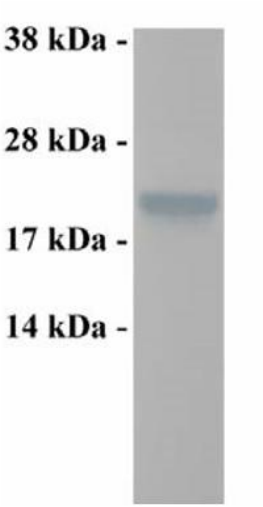

Figure 2. Rap1 positive control (10 µL of NIH3T3 cell lysate at 0.75 mg/mL) detected by immunoblot.

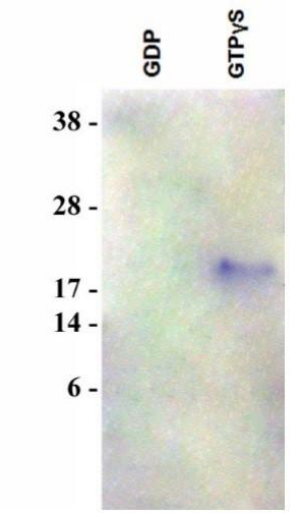

Figure 3. Rap1 activation assay. Lane 1: NIH 3T3 cell lysate loaded with GDP and incubated with RalGDS RBD Agarose beads. Lane 2: NIH 3T3 cell lysate loaded with GTPγS and incubated with RalGDS RBD Agarose beads.

13. Troubleshooting

Problem	Solution
No Rap1 signal detected from Immunoblot control	Use a freshly diluted batch of Rap1 antibody
	Ensure transfer procedure is successful (use Ponceau-S to check protein transfer to membrane)
No Rap1 signal from cells (including GTPγS control)	Ensure you are able to detect Rap1 in your sample – perform a western blot directly on your cell lysate using the antibody supplied in the kit without performing the pull-down.
	Use as much lysate as possible. Since active form is usually a very small percentage of the total. We recommend using 100 times the lysate amount needed to see a clearly defined band in the direct western blot (see above)
Rap1 signal detected in GTPγS control but not in stimulated cells	Use a different batch of agonist/stimulus or check they are inducing Rap1 in your sample
	In vivo stimulation of cells will activate approximately 10% of available Rap1, whereas *in vitro* GTPγS loading will activated nearly 90% of Rap1
Additional unknown higher MW bands detected	Ensure species is recognized by antibody – this antibody specifically reacts with human, mouse and rat Rap1
	Perform a western blot directly on your crude cell lysate using the antibody to confirm/discard non-specific binding

14. FAQs

Q. Why can't I use conventional western blotting to detect the active form of the small GTPase?

A. Strictly relying on western blot for detecting any active small GTPase is not recommended. We are not aware of any antibody that will reliably bind the GTP (active) form and not the GDP (inactive) form.

Q. Can I use a different lysis buffer?

A. Yes, other lysis buffers, such as RIPA buffer, can be used for sample lysate preparation.

Q. Can I run the controls just once for the assay or do I need to run them with each sample?

A. The GDP (negative) and GTPγS (positive) loading controls to verify that the pull down was performed correctly. Since the controls are independent of the endogenous small GTPase activity of your samples, they only need to be run once.

15. Notes

Technical Support

For all technical or commercial enquiries please go to:
https://www.abcam.com/en-us/contact-us
https://www.abcam.cn/contact-us (China)
https://www.abcam.co.jp/contact-us (Japan)