# Measuring GTPase, GEF and GAP Activities Using the GTPase-Glo™ Assay and the GloMax® Discover System

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**Materials Required**

- GTPase-Glo™ Assay (Cat.# V7681 and V7682)
- GloMax® Discover System (Cat.# GM3000)
- white, 96-well half-area assay plates (Corning Cat.# 3693)
- Nuclease-Free Water (Cat.# P1195)
- Ras wild type (Millipore Cat.# 553325)
- $Ras^{G12V}$ mutant (Jena Biosciences Cat.# PR-206)
- NF1-333 (Jena Biosciences Cat.# PR-223)

**Caution:** We recommend the use of gloves, lab coats and eye protection when working with these or any chemical reagents.

**Protocols:** *GloMax® Discover System Technical Manual* #TM397 and *GTPase-Glo™ Assay Technical Manual* #TM452 are available at: **www.promega.com/protocols/**

GTPases play a major role in various cellular functions such as cell signaling, cell proliferation, cell differentiation, cytoskeleton modulation and cell motility. Deregulation or mutation of these proteins can result in serious pathological conditions.

GTPases comprise a large family of enzymes that bind and hydrolyze guanine triphosphate (GTP). The GTP binding and hydrolysis allows them to act as molecular switches for signal transduction, and cycle between an activated GTP-bound state and an inactive GDP-bound state. GTPases have a high affinity for guanine nucleotides GDP or GTP and are slow-acting GTP hydrolases.

To mediate the process of shuttling between the active and inactive forms, GTPases need the help of two families of proteins: GTPase-activating proteins (GAPs) and guanine nucleotide exchange factors (GEFs). GEFs replace the GDP bound to inactive GTPase with GTP, making the enzyme active and transducing a wide variety of cellular signals. GTPases then require the help of GAPs to hydrolyze GTP and switch off the signalling cascade.

Targeting GTPases and their regulators can be challenging due to lack of convenient assays. The GTPase-Glo™ Assay consists of optimized reaction buffers that allow continuous progression of the GTPase cycle and hydrolysis of GTP. The GTPase/GAP buffer is optimized for measuring intrinsic GTPase and GAP-mediated GTPase activities while the GEF Buffer is optimized for performing reactions mediated by GEFs in the presence of GTPase and GAP. The assay relies on enzymatic conversion of GTP remaining after the GTPase reaction into ATP followed by bioluminescent detection of the created ATP. This sensitive assay analyzes the intrinsic activities of GTPase, GAP-stimulated GTPase, GAP and GEF with minimal false hits when tested for compound interference using the library of pharmacologically active compounds (LOPAC). Thus the GTPase-Glo™ Assay can be used to identify small molecule modulators of GTPase during high-throughput screening.

Measuring the luminescence from the GTPase-Glo™ Assay is easy on the GloMax® Discover System because the protocol comes preloaded on the instrument. The extended dynamic range and minimal well-to-well cross talk of the GloMax® Discover System allows you to easily measure signals of varying intensities on the same plate. This Application Note describes the protocol to measure GAP-stimulated GTPase activity using the GTPase-Glo™ Assay and GloMax® Discover System.

## GTPase-Glo™ Assay Protocol

For detailed instructions and assay notes for various assay volumes and plate formats, see the *GTPase-Glo™ Assay Technical Manual* #TM452. The following protocol is performed in 384-well plates.

1. Prepare a 2X GTPase-GTP solution containing 1mM DTT and GTPase (Ras wild type or $Ras^{G12V}$) at twice the desired final concentration in GTPase/GAP Buffer. Dispense 5µl into wells of a 384-well plate.
2. Assemble and initiate the GTPase reaction by adding 5µl of the GAP NF1-333 and GTP, such that the final concentration of NF1-333 is 1µM and GTP is 5µM in a total reaction volume of 10µl. Include a sample containing 5µM GTP in GTPase/GAP Buffer as a no-enzyme control.
3. Incubate the reaction at room temperature (22–25°C) for the optimal time, generally 60–120 minutes.

   **Note:** During the incubation, thaw the undiluted GTP-Glo™ Reagent at room temperature until ready to use in Step 5. Calculate the volume of GTPase-Glo™ Reagent, 500X, required for the experiment.
4. Gently mix the thawed GTPase-Glo™ Reagent, 500X, by inversion; do not vortex. Prepare the required volume of reconstituted GTPase-Glo™ Reagent by increasing or decreasing the component volumes provided. For example, make 1ml of reconstituted GTPase-Glo™ Reagent by adding 2µl of GTPase-Glo™ Reagent, 500X, and 0.5µl of ADP, 10mM, to 998µl of GTPase-Glo™ Buffer.

   **Note:** Prepare the reconstituted GTPase-Glo™ Reagent immediately before use, and prepare only enough for your experiment. Do not freeze the reconstituted GTPase-Glo™ Reagent.
5. Add 10µl of reconstituted GTPase-Glo™ Reagent to the completed GTPase reaction, mix briefly and incubate with shaking for 30 minutes at room temperature (22–25°C).
6. Add 20µl of Detection Reagent, and incubate the plate for 5–10 minutes at room temperature (22–25°C).
7. Measure luminescence (GTPase, GAP or GEF activity) on the GloMax® Discover System by selecting "GTPase-Glo" from the list of preset protocols.

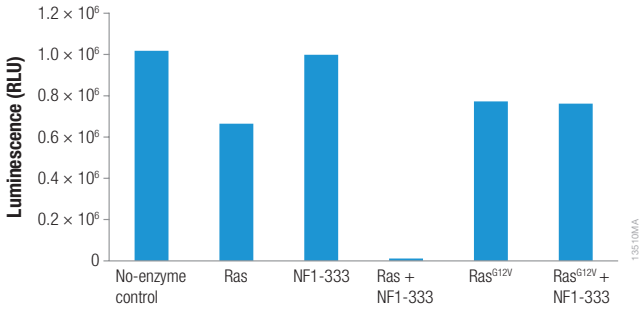


**Figure 1. GTPase activity and GAP-mediated GTPase activity of Ras and NF1-333.** Reactions were assembled with 2µM wildtype or mutant Ras, 1µM NF1-333 and 5µM GTP in GTPase/GAP Buffer containing 1mM DTT. The final reaction volume was 10µl. Reactions were incubated for 90 minutes at room temperature. To the completed GTPase reactions, 10µl of GTPase-Glo™ Reagent was added and incubated for 30 minutes at room temperature. Twenty microliters of Detection Reagent was added, plates were incubated for 5–10 minutes at room temperature and luminescence was recorded using the GloMax® Discover System.

## Conclusion

The GloMax® Discover can detect luminescence generated using the GTPase-Glo™ Assay as shown in Figure 1. Luminescence for the no-enzyme control (buffer only) represents the total amount of input GTP. A decrease in light output, which represents the intrinsic GTPase activity, was observed when Ras was included in the reaction. NF1-333 alone does not possess any GTPase activity, but in the presence of Ras, almost all of the input GTP was hydrolyzed. Note that the constitutively activated oncogenic mutant $Ras^{G12V}$ does not show GAP-stimulated GTPase activity.

## The GloMax® Discover System

The GloMax® Discover System offers superior sensitivity and dynamic range and limited well-to-well cross talk. The instrument was developed and optimized with Promega cell and gene reporter assays and may be integrated into low- and medium-throughput automation workflows. The GloMax® Discover System allows flexible use of filters to measure fluorescence intensity, filtered luminescence, BRET, FRET and UV-visible absorbance for a wide variety of laboratory applications. The instrument is operated by an integrated Tablet PC, which provides quick and easy navigation through the control options. Exporting your results is made seamless with a variety of options, including exporting data to your local network.