# Bioluminescent Assay for GTPases Allows Measurement of GTPase, GAP and GEF Activities

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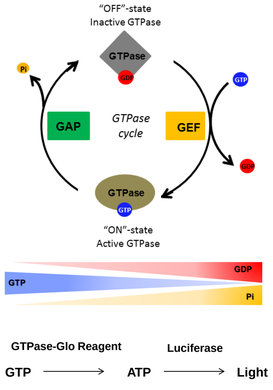
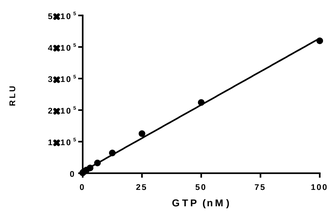
## 1. Introduction

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 results in in serious pathological conditions. Targeting GTPases and their regulators have been challenging due to lack of convenient assays. To overcome the challenges in analyzing activities of GTPases and their regulatory proteins GTPase Activating Proteins (GAP) and Guanine Nucleotide Exchange Factors (GEF) we have developed a homogenous bioluminescent assay (GTPase-Glo) system to analyze these proteins in a simple, convenient "add-mix-read" format.

The assay consists of optimized reaction buffers that allow continuous progression of the GTPase cycle and hydrolysis of GTP. The assay relies on enzymatic conversion of GTP remaining after the GTPase reaction to ATP and bioluminescent detection of the ATP. We show that the assay is sensitive and robust when analyzing for analyzing intrinsic GTPase- activity, GAP-stimulated GTPase- activity, GAP- activity and GEF- activity. The assay has minimal false hits when tested for compound interference using the LOPAC library (library of pharmacologically active compounds) indicative of the robustness in identifying small molecule modulators of GTPase using high throughput screening

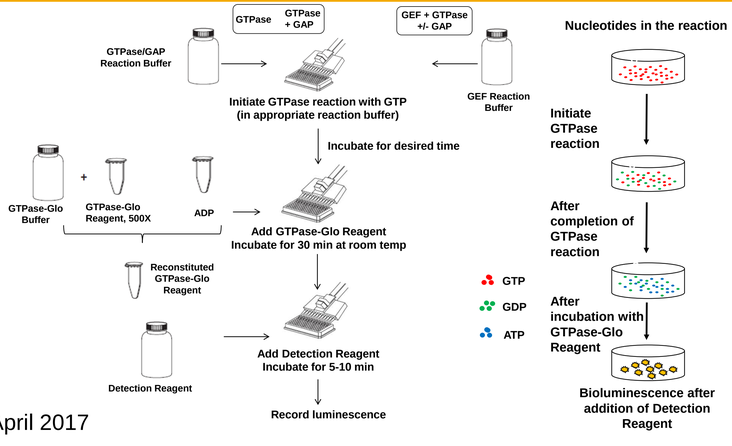
## 2. Assay Principle



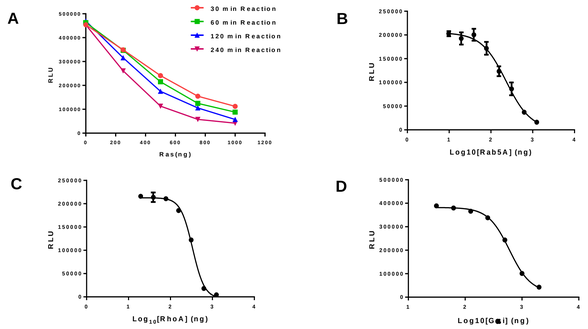




- Optimized reaction buffers allow GTPase reaction to continually cycle allowing GTP hydrolysis.
- GTP remaining after the completion of the GTPase reaction is converted to ATP by an enzyme coupled reaction.
- ATP is detected by a bioluminescent readout.
- There is an inverse correlation between light output and GTPase activity.

## 3. Assay Procedure

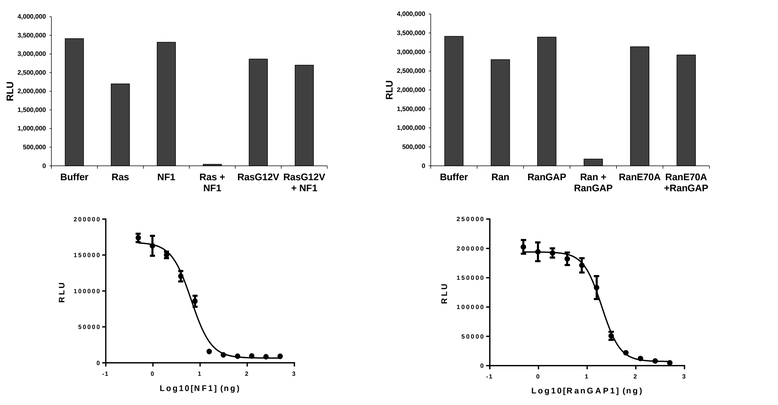



## 4. Intrinsic GTPase Activity

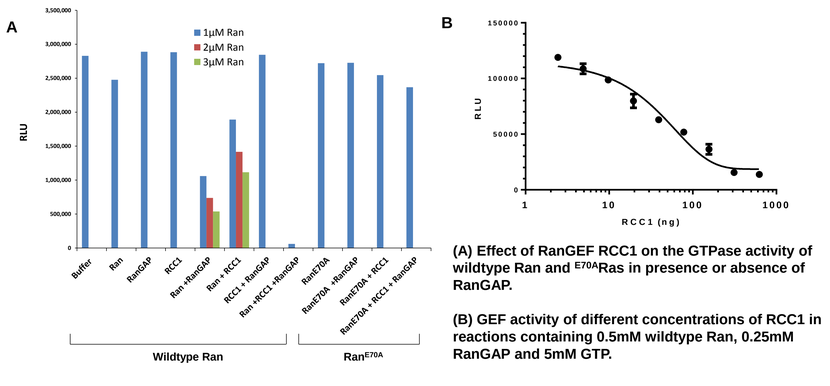



**(A) Intrinsic GTPase activity of Ras at different concentration for different times. Intrinsic GTPase activities at different concentrations of (B) Rab5A, (C) RhoA, (D) $G_{\alpha i}$.**

## 5. GAP-Stimulated GTPase Activity



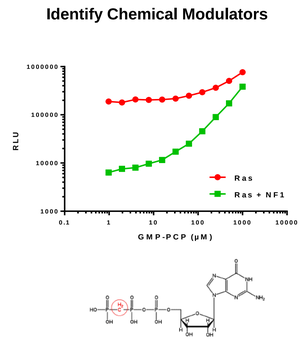

## 6. GEF Activity

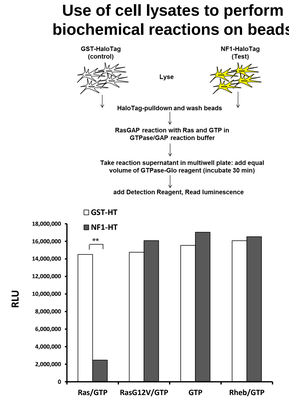



**(A) Effect of RanGEF RCC1 on the GTPase activity of wildtype Ran and $^{E70A}$Ras in presence or absence of RanGAP.**

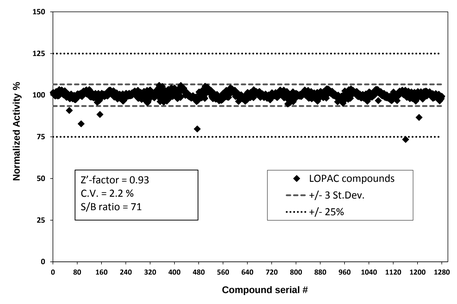
**(B) GEF activity of different concentrations of RCC1 in reactions containing 0.5mM wildtype Ran, 0.25mM RanGAP and 5mM GTP.**

## 7. Applications of the bioluminescent GTPase Assay




## 8. LOPAC Screening




**GTP was detected using GTPase-Glo™ Assay in presence of LOPAC compounds at a final compound concentration of 10µM in DMSO. There were only two compounds which were known luciferase inhibitors that showed 25% inhibition of the assay validating the assay for high throughput screening. Signal: background ratio of 71. The normalized data after background subtraction is represented.**

## 9. Conclusions

- **Universal:** The assay can be used to measure the activities of GTPases (small GTPases and hetero-trimeric GTPases), GTPase Activating Proteins (GAPs) and Guanine Nucleotide Exchange Factors (GEFs).
- **Highly sensitive:** Allowing use of low amounts of proteins in an assay.
- **Easy to use:** The assay is performed in a convenient "add-mix-and-read" format and the assay readout is achieved in 30 minutes after the completion of the GTPase reaction.
- **HTS compatible:** The homogenous nature of the assay will allow screening of chemical modulators of GTPases, GAPs and GEFs.
- **Pulldown-based capability using cell lysates:** By combining the assay procedure to pulldown enzymes of interest, GTPase/GAP/GEF activity can be analyzed in cell lysates.



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