

## RhoGEF exchange assay Biochem Kit™

(Cat. # BK100)

The Ras superfamily of small GTPases (such as Ras, Rho, Rab, Arf and Ran proteins) serve as binary switches cycling between a GDP-bound “OFF state” and a GTP-bound “ON state”. In cells, the cycling between the two states is mainly controlled by two types of regulatory proteins: the activating Guanine nucleotide Exchange Factors (GEFs) and the inactivating GTPase Activating Proteins (GAPs).

GEFs function by catalyzing the exchange of nucleotide on their target GTPases. The GEF will bind to a nucleotide-bound GTPase, which causes the bound nucleotide to be released, thus resulting in a nucleotide free GEF-GTPase reaction intermediate. This nucleotide free complex will then take up a new nucleotide after which the GEF is released from the GTPase. Because GEFs typically have a higher affinity for GDP-bound GTPases than for the corresponding GTP-bound GTPase and the intracellular ratio of GTP to GDP is about 10:1, GEFs will drive the exchange from GDP-bound GTPases to GTP-bound GTPases



The fluorophore based assay in this kit is suitable for measuring nucleotide exchange on GTPases in either 96-well or 384-well format. The kit measures the uptake of the fluorescent nucleotide analog N-methylanthraniloyl-GTP (mant-GTP) into GTPases. The uptake can be measured due to the spectroscopic difference between free and GTPase-bound mant-GTP. As mant-GTP gets bound in the nucleotide binding pocket of a GTPase, its fluorescence (ex: 360 nm, em: 440 nm) increases dramatically. Therefore, the enhancement of mant-GTP fluorescent intensity in the presence of a small GTPase indicates nucleotide uptake (or exchange for already bound nucleotide) by the GTPase.

This kit contains all the buffers and reagents needed for the assay, a 96 and a 384-well plate, as well as human Cdc42, Rac1 and RhoA GTPases and the GEF domain of Dbs as a positive control GEF. Dbs is an effective GEF for Cdc42 and RhoA but shows only very low GEF activity for Rac1 (see Figs 1 & 2). The kit provides enough material for 20 assays for each GTPase in 96-well format or 130 assays for each GTPase in 384-well format.

While the kit comes with Cdc42, Rac1 and RhoA proteins, it can also be used for any other Ras superfamily GTPase. See our [G-protein product family](#) web page for other GTPases available from Cytoskeleton.

For a kit to measure GAP activity, see Cat. # [BK105](#)

**Uses:**

1. Determination of the activity and GTPase specificity of uncharacterized GEFs.
2. Biochemical characterization of small GTPases and their associated GEFs.
3. Examination of the regulation of GEF activity by different cofactors or protein domains.
4. Screen the mutant protein of either GEFs or GTPases for activity and substrate specificity.
5. Identification of GEF inhibitors in HTS (high throughput screen) format. Please inquire for significant discounts on large quantities of any reagents in this kit

**Contents:**

- 1) Exchange Buffer (2 x)
- 2) 100 µg His-tagged Cdc42 protein (Cat. # CD01)
- 3) 100 µg His-tagged Rac1 protein (Cat. # RC01)
- 4) 100 µg His-tagged RhoA protein (Cat. # RH01)
- 5) 50 µg His-tagged hDbs protein (Cat. # GE01)
- 6) 384 well black half area round bottom plate
- 7) 96 well black half area flat bottom plate
- 8) Detailed instructions and troubleshooting manual.

**Example Results:**

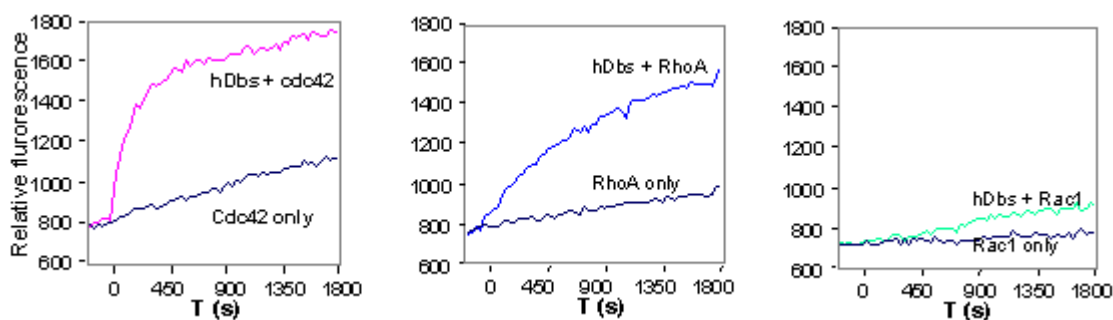


Figure 1. Dbs exchange activity for Cdc42, RhoA and Rac1 in 96-well half area plate format

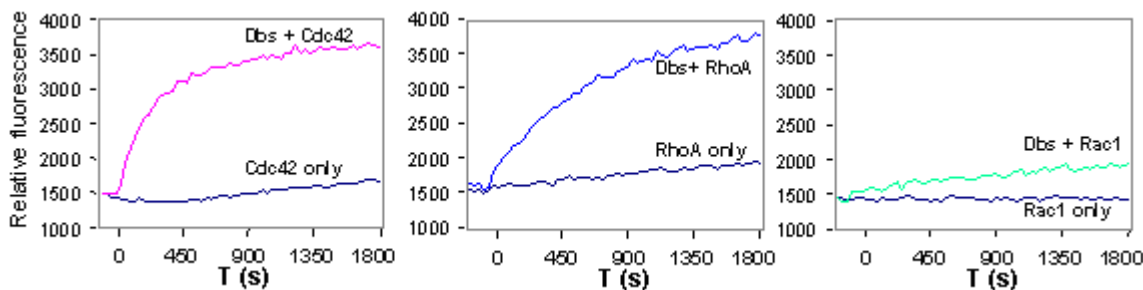


Figure 2. Dbs exchange activity for Cdc42, RhoA and Rac1 in 384-well plate format

Catalog Number	Amount	
BK100	1 kit (60-300 assays)	_____
BK100-HTS	Large Quantities	_____