

Product datasheet

ROCK Activity Assay Kit ab211175

[3 References](#) [1 Image](#)

Overview

Product name	ROCK Activity Assay Kit
Detection method	Colorimetric
Sample type	Cell Lysate, Tissue Lysate
Sensitivity	0.2 ng
Species reactivity	Reacts with: Human
Product overview	ROCK Activity Assay Kit (ab211175) provides a non-isotopic, sensitive and specific method to monitor ROCK1 (ROK β) and ROCK2 (Rho Kinase) using its physiological substrate MYPT1 (myosin phosphate target subunit 1). This kit can also be used in the screening of Rho kinase inhibitor molecules in purified protein samples.

This product detects the specific phosphorylation of MYPT1 by ROCK1 and ROCK2 at threonine 696 (T696) using an enzyme-based immunoassay. This substrate is initially incubated with the sample containing ROCK1 and ROCK2 (such as purified kinase or cell lysate). Following the kinase reaction, the sample solution is transferred to a substrate capture plate where phosphorylated MYPT1 is detected by anti-phospho-MYPT1-Thr696 antibody. A recombinant active ROCK2 is provided as a positive control. The sensitivity limit of this kit is 200 pg of active ROCK2.

Notes	Members of the Rho family are essential regulatory components of the signaling pathway that direct cell motility, adhesion, and cytokinesis through reorganization of actin cytoskeleton. Rho is activated by extracellular signals such as lysophosphatidic acid (LPA). The actions of Rho are mediated by downstream Rho effectors. One of these effectors is Rho-associated kinase (ROCK). Two ROCK isoforms have been identified: ROCK1 (also known as ROK β) and ROCK2 (also known as Rho Kinase and ROK α). ROCK mediates Rho signaling and reorganizes actin cytoskeleton through phosphorylation of several substrates that contribute to the assembly of actin filaments and contractility. For example, ROCK inactivates myosin phosphatase through the specific phosphorylation of myosin phosphatase target subunit 1 (MYPT1) at Thr696, which results in an increase in the phosphorylated content of the 20-kDa myosin light chain (MLC20).
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Properties

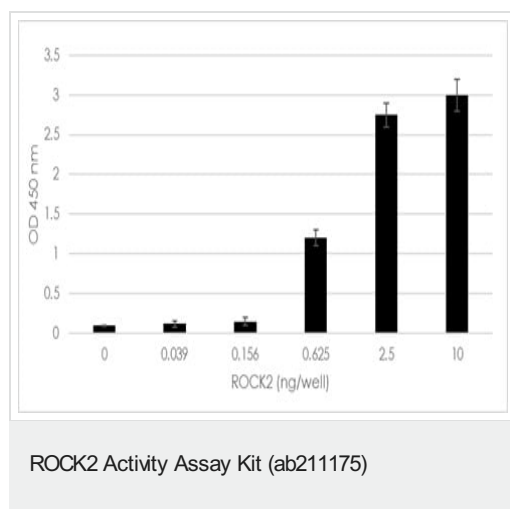
Storage instructions	Please refer to protocols.
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Components	1 x 96 tests	5 x 96 tests
10X Kinase Buffer	1 x 20ml	5 x 20ml
10X Wash Buffer	1 x 100ml	5 x 100ml
Active ROCK-II (0.5 µg/mL)	1 x 20µl	5 x 20µl
Anti-Phospho-MYPT1 (Thr696) antibody	1 x 20µl	5 x 20µl
Assay Diluent	1 x 50ml	5 x 50ml
ATP Solution (100 mM)	1 x 100µl	5 x 100µl
Rho Kinase Substrate Coated Plate	1 x 96 tests	5 x 96 tests
Secondary Antibody, HRP Conjugate	1 x 20µl	5 x 20µl
Stop Solution	1 x 12ml	5 x 12ml
Substrate Solution	1 x 12ml	5 x 12ml

Cellular localization

ROCK2: Cytoplasm. Cell membrane. Cytoplasmic, and associated with actin microfilaments and the plasma membrane. ROCK1: Cytoplasm. Cytoplasm, cytoskeleton, microtubule organizing center, centrosome, centriole. Golgi apparatus membrane. Cell projection, bleb. Cytoplasm, cytoskeleton. Cell membrane. Cell projection, lamellipodium. Cell projection, ruffle. Associated with the mother centriole and an intercentriolar linker. Colocalizes with ITGB1BP1 and ITGB1 at the cell membrane predominantly in lamellipodia and membrane ruffles, but also in retraction fibers. Localizes at the cell membrane in an ITGB1BP1-dependent manner (By similarity). A small proportion is associated with Golgi membranes.

Images



ROCK2 Kinase assay. Increasing amounts of ROCK2 were incubated for 60 minutes at 30°C. Phosphorylated MYPT1 was detected with specific anti-phospho-MYPT1 (T696) as described in the assay protocol.

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