Rho-kinase (Human) Assay Kit

Cat. No. Kit-0767 **Lot. No.** (See product label)

SPECIFICATION

Product Overview	Rho-kinase (Human) Assay Kit is a single-site, non-quantitative immunoassay for Rho-kinase activity. Plates are pre-coated with a substrate corresponding to recombinant the C terminus of MBS (Myosin-Binding Subunit of myosin phosphatase), which contains a threonine residue that may be phosphorylated by DMPK family members, including Rho-kinase (ROCK1 and ROCK2), MRCK (Myotonic Dystrophy kinase-related Cdc42-binding Kinase) and DMPK (Myotonic Dystrophy Protein Kinase). The detector antibody is AF20, an antibody that specifically detects only the phosphorylated form of threonine-696 on MBS.
Description	The small GTPase Rho regulates formation of focal adhesions and stress fibers of fibroblasts, as well as adhesion and aggregation of platelets and lymphocytes by shuttling between the inactive GDP-bound form and the active GTP-bound form. Rho is also essential in cytokinesis and plays a role in transcriptional activation by serum response factor. Ishizaki et al. (1996) identified the protein serine/threonine kinase ROCK1 (Rho-kinase beta), which they called p160-ROCK, which is activated when bound to the GTP-bound form of RhoA. Fujisawa et al. (1996) localized the Rho-binding domain of ROCK1 to a region between residues 934-1015.ROCK2 (Rho-kinase alpha) is a serine/threonine kinase that regulates cytokinesis, smooth muscle contraction, the formation of actin stress fibers and focal adhesions, and the activation of the c-fos serum response element. ROCK2, which is an isozyme of ROCK1, is a target for the small GTPase Rho. Nakamura et al. (2001) studied the role of Rho in the migration of corneal epithelial cells in rabbit. They detected both ROCK1 and ROCK2 in the corneal epithelium at protein and mRNA levels. They found that exoenzyme C3, a Rho inhibitor, inhibits corneal epithelial migration in a

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	dose-dependent manner and prevents the stimulatory effect of the Rho activator lysophosphatidic acid (LPA). Both cytochalasin B, an inhibitor of actin filament assembly, and ML7, an inhibitor of myosin light chain kinase, also prevent LPA stimulation of epithelial migration. The authors suggested that Rho mediates corneal epithelial migration in response to external stimuli by regulating the organization of the actin cytoskeleton.
Applications	1) Monitoring the purification of Rho-kinase or DMPK family kinase.2) Screening inhibitors or activators of Rho-kinase or DMPK family kinase.3) Detecting the effects of pharmacological agents on Rho-kinase or DMPK family kinase.
Target Species	Human
Usage	For research use only (RUO)
Storage	• Upon receipt store the ATP at 4°C• Do not expose reagents to excessive light
Kit Components	Microplate: One microplate supplied ready to use, with 96 wells (12 strips of 8-wells) in a foil, zip-lock bag with a desiccant pack. Wells are coated with recombinant MBS C-terminus (654-880 a.a.) as Rho-kinase substrate.10X Wash Buffer: One 100 mL bottle of 10X buffer containing 2%Tween -20Kinase Buffer: One bottle containing 20 mL of 1X buffer; used for Kinase Reaction Buffer and sample dilution.20X ATP: Lyophilized ATP Na2 salt. Reconstitute contents of vial with 0.8 mL of H2O.HRP conjugated Detection Antibody: One vial containing 12 mL of HRP (horseradishperoxidase) conjugated anti-phospho-MBS T696 (AF20) antibody. Ready to use.Substrate Reagent: 20 mL of the chromogenic substrate, tetra-methylbenzidine (TMB). Ready to use.Stop Solution: One bottle supplied ready to use, containing 20 mL of 1 N H2SO4.

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