#13430 Store at -20°C

Focal Adhesion Protein Antibody Sampler Kit

☑ 1 Kit
(6 x 20 µl)

Cell Signaling TECHNOLOGY®

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For Research Use Only. Not For Use In Diagnostic Procedures.

Products Included	Product #	Quantity	Mol. Wt.	Isotype
α-Actinin (D6F6) XP® Rabbit mAb	6487	20 µl	100 kDa	Rabbit IgG
FAK Antibody	3285	20 µl	125 kDa	Rabbit IgG
Paxillin (D9G12) Rabbit mAb	12065	20 µl	54, 62, 68 kDa	Rabbit IgG
Talin-1 (C45F1) Rabbit mAb	4021	20 µl	270 kDa	Rabbit IgG
Tensin 2 Antibody	11990	20 µl	145-155 kDa	Rabbit IgG
Vinculin Antibody	4650	20 µl	124 kDa	Rabbit IgG
Anti-rabbit IgG, HRP-linked Antibody	7074	100 µl		Goat

See www.cellsignal.com for individual component applications, species cross-reactivity, dilutions and additional application protocols.

Storage: Supplied in 10 mM sodium HEPES (pH 7.5), 150 mM NaCl, 100 µg/ml BSA, 50% glycerol and less than 0.02% sodium azide. Store at –20°C. *Do not aliquot the antibodies.*

Recommended Antibody Dilutions:
Western blotting 1:1000

Please visit www.cellsignal.com for validation data and a complete listing of recommended companion products.

Description: The Focal Adhesion Protein Antibody Sampler Kit provides an economical means to evaluate proteins involved in focal adhesions. The kit includes enough antibody to perform two western blot experiments per primary antibody.

Background: Focal adhesions connect the cytoskeleton with the extracellular matrix (ECM), a complex structure of secreted macromolecules that surrounds mammalian organs and tissues. Integrins clustered on the extracellular side of focal adhesions relay signals from the ECM to intracellular protein complexes that signal the actin cytoskeleton to regulate tension for cell motility. Internal signals converge on focal adhesions to regulate integrin receptor affinity and avidity. Signaling through focal adhesions regulates cell adhesion, migration, proliferation, apoptosis, and gene expression, and impacts cellular processes such as development, wound healing, immune response, invasion, metastasis and angiogenesis (reviewed in 1-3). Focal adhesion kinase (FAK) is a widely expressed cytoplasmic protein tyrosine kinase involved in integrin-mediated signal transduction. Integrin clustering triggers FAK recruitment to the focal adhesion complex (4). Talin is a large, multidomain focal adhesion protein that interacts with the intracellular domains of integrins and other focal adhesion proteins. Talin is involved in the formation of focal adhesions and in linking focal adhesions to the actin cytoskeleton (5). Paxillin is a key component of integrin signaling that localizes primarily to focal adhesion sites in the extracellular matrix (6). Tyrosine phosphorylation of paxillin is required for integrin-mediated cytoskeletal reorganization (7). Paxillin is phosphorylated by FAK at Tyr118 (8,9). Vinculin is a cytoskeletal protein involved in regulation of focal adhesions and embryonic development (10-13). Active vinculin translocates to focal adhesions where it may be involved in anchoring F-actin to the membrane and regulating cell migration. Vinculin binds a number of proteins, including talin, α-actinin and paxillin (11,13). Tensin 2 localizes to focal adhesions of various tissues and exhibits highest expression in heart, kidney, and liver (14,15). Tensin 2 belongs to a family of cytoskeletal proteins that include Tensin 1-3 and Cten, which couple integrins to the actin cytoskeleton (16). Tensin family proteins play an important role in signal transduction, cell proliferation, and motility (17-20). α-actinin is a member of the spectrin family of cytoskeletal proteins that was first recognized as an actin cross-linking protein, but also interacts with a large number of cytoskeletal signaling proteins, including those involved in cellular adhesion, migration, and immune cell targeting (21).

Specificity/Sensitivity: Each antibody in this kit recognizes endogenous total levels of its specific target protein. Paxillin (D9G12) Rabbit mAb recognizes endogenous levels of total paxillin protein and recognizes all human paxillin isoforms. Vinculin Antibody detects endogenous levels of total vinculin protein and also reacts with metavinculin, a 145 kDa splice variant of vinculin.

Source/Purification: Monoclonal antibodies are produced by immunizing animals with a synthetic peptide corresponding to residues surrounding Phe316 of human α-actinin protein, surrounding Lys193 of human paxillin protein, and near the carboxy terminus of human talin-1 protein. Polyclonal antibodies are produced by immunizing animals with a synthetic peptide corresponding to residues surrounding Glu710 of human FAK protein, surrounding Arg1555 of human tensin 2 protein, and residues near

Background References:

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Applications Key: **W**—Western **IP**—Immunoprecipitation **IHC**—Immunohistochemistry **ChIP**—Chromatin Immunoprecipitation **IF**—Immunofluorescence **F**—Flow cytometry **E-P**—ELISA-Peptide

Species Cross-Reactivity Key: **H**—human **M**—mouse **R**—rat **Hm**—hamster **Mk**—monkey **Mi**—mink **C**—chicken **Dm**—D. melanogaster **X**—Xenopus **Z**—zebrafish **B**—bovine **Dg**—dog **Pg**—pig **Sc**—S. cerevisiae **All**—all species expected Species enclosed in parentheses are predicted to react based on 100% homology.

#13430

Western Immunoblotting Protocol

For western blots, incubate membrane with diluted primary antibody in either 5% w/v BSA or nonfat dry milk, 1X TBS, 0.1% Tween® 20 at 4°C with gentle shaking, overnight.
NOTE: Please refer to primary antibody datasheet or product webpage for recommended primary antibody dilution buffer and recommended antibody dilution.

A. Solutions and Reagents

NOTE: Prepare solutions with reverse osmosis deionized (RODI) or equivalent grade water.

1. **20X Phosphate Buffered Saline (PBS):** (#9808) To prepare 1 L 1X PBS: add 50 ml 20X PBS to 950 ml dH_2O, mix.
2. **10X Tris Buffered Saline (TBS):** (#12498) To prepare 1 L 1X TBS: add 100 ml 10X to 900 ml dH_2O, mix.
3. **1X SDS Sample Buffer:** Blue Loading Pack (#7722) or Red Loading Pack (#7723) Prepare fresh 3X reducing loading buffer by adding 1/10 volume 30X DTT to 1 volume of 3X SDS loading budder. Dilute to 1X with dH_2O.
4. **10X Tris-Glycine SDS Running Buffer:** (#4050) To prepare 1 L 1X running buffer: add 100 ml 10X running buffer to 900 ml dH_2O, mix.
5. **10X Tris-Glycine Transfer Buffer:** (#12539) To prepare 1 L 1X Transfer Buffer: add 100 ml 10X Transfer Buffer to 200 ml methanol + 700 ml dH_2O, mix.
6. **10X Tris Buffered Saline with Tween® 20 (TBST):** (#9997) To prepare 1 L 1X TBST: add 100 ml 10X TBST to 900 ml dH_2O, mix.
7. **Nonfat Dry Milk:** (#9999)
8. **Blocking Buffer:** 1X TBST with 5% w/v nonfat dry milk; for 150 ml, add 7.5 g nonfat dry milk to 150 ml 1X TBST and mix well.
9. **Wash Buffer:** (#9997) 1X TBST
10. **Bovine Serum Albumin (BSA):** (#9998)
11. **Primary Antibody Dilution Buffer:** 1X TBST with 5% BSA or 5% nonfat dry milk as indicated on primary antibody datasheet; for 20 ml, add 1.0 g BSA or nonfat dry milk to 20 ml 1X TBST and mix well.
12. **Biotinylated Protein Ladder Detection Pack:** (#7727)
13. **Prestained Protein Marker, Broad Range (Premixed Format):** (#7720)
14. **Blotting Membrane and Paper:** (#12369) This protocol has been optimized for nitrocellulose membranes. Pore size 0.2 µm is generally recommended.
15. **Secondary Antibody Conjugated to HRP:** anti-rabbit (#7074); anti-mouse (#7076)
16. **Detection Reagent:** LumiGLO® chemiluminescent reagent and peroxide (#7003) or SignalFire™ ECL Reagent (#6883)

B. Protein Blotting

A general protocol for sample preparation.

1. Treat cells by adding fresh media containing regulator for desired time.
2. Aspirate media from cultures; wash cells with 1X PBS; aspirate.
3. Lyse cells by adding 1X SDS sample buffer (100 µl per well of 6-well plate or 500 µl for a 10 cm diameter plate). Immediately scrape the cells off the plate and transfer the extract to a microcentrifuge tube. Keep on ice.
4. Sonicate for 10–15 sec to complete cell lysis and shear DNA (to reduce sample viscosity).
5. Heat a 20 µl sample to 95–100°C for 5 min; cool on ice.
6. Microcentrifuge for 5 min.
7. Load 20 µl onto SDS-PAGE gel (10 cm x 10 cm). **NOTE:** Loading of prestained molecular weight markers (#7720, 10 µl/lane) to verify electrotransfer and biotinylated protein ladder (#7727, 10 µl/lane) to determine molecular weights are recommended.
8. Electrotransfer to nitrocellulose membrane (#12369).

C. Membrane Blocking and Antibody Incubations

NOTE: Volumes are for 10 cm x 10 cm (100 cm^2) of membrane; for different sized membranes, adjust volumes accordingly.

I. Membrane Blocking

1. (Optional) After transfer, wash nitrocellulose membrane with 25 ml TBS for 5 min at room temperature.
2. Incubate membrane in 25 ml of blocking buffer for 1 hr at room temperature.
3. Wash three times for 5 min each with 15 ml of TBST.

II. Primary Antibody Incubation

1. Incubate membrane and primary antibody (at the appropriate dilution and diluent as recommended in the product datasheet) in 10 ml primary antibody dilution buffer with gentle agitation overnight at 4°C.
2. Wash three times for 5 min each with 15 ml of TBST.
3. Incubate membrane with the species appropriate HRP-conjugated secondary antibody (#7074 or #7076 at 1:2000) and anti-biotin, HRP-linked Antibody (#7075 at 1:1000–1:3000) to detect biotinylated protein markers in 10 ml of blocking buffer with gentle agitation for 1 hr at room temperature.
4. Wash three times for 5 min each with 15 ml of TBST.
5. Proceed with detection (Section D).

D. Detection of Proteins

1. Incubate membrane with 10 ml LumiGLO® (0.5 ml 20X LumiGLO® #7003, 0.5 ml 20X Peroxide, and 9.0 ml purified water) or 10 ml SignalFire™ #6883 (5 ml Reagent A, 5 ml Reagent B) with gentle agitation for 1 min at room temperature.
2. Drain membrane of excess developing solution (do not let dry), wrap in plastic wrap and expose to x-ray film. An initial 10 sec exposure should indicate the proper exposure time. **NOTE:** Due to the kinetics of the detection reaction, signal is most intense immediately following incubation and declines over the following 2 hr.