

# Vav Isoform Antibody Sampler Kit

✓ 1 Kit  
(3 x 40 µl)

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This product is intended for research purposes only. This product is not intended to be used for therapeutic or diagnostic purposes in humans or animals.

Products Included	Product #	Quantity	Mol. Wt.	Isotype
Vav1 (D45G3) Rabbit mAb	4657	40 µl	95 kDa	Rabbit IgG
Vav2 (C64H2) Rabbit mAb	2848	40 µl	100 kDa	Rabbit IgG
Vav3 Antibody	2398	40 µl	98 kDa	Rabbit IgG
Anti-rabbit IgG, HRP-linked Antibody	7074	100 µl		Goat

See [www.cellsignaling.com](http://www.cellsignaling.com) for individual component applications, species cross-reactivity, dilutions and additional application protocols.

**Description:** The Vav Isoform Antibody Sampler Kit is an economical way to examine the total protein levels of Vav isoforms 1, 2, and 3. The kit includes enough primary and secondary antibodies to perform four Western blot experiments.

**Background:** Vav proteins belong to the Dbl family of guanine nucleotide exchange factors (GEFs) for Rho/Rac small GTPases. The three identified mammalian Vav proteins (Vav1, Vav2 and Vav3) differ in their expression. Vav1 is expressed only in hematopoietic cells and is involved in the formation of the immune synapse. Vav2 and Vav3 are more ubiquitously expressed. Vav proteins contain the Dbl homology domain, which confers GEF activity, as well as protein interaction domains that allow them to function in pathways regulating actin cytoskeleton organization (reviewed in 1). Phosphorylation stimulates the GEF activity of Vav protein towards Rho/Rac (2,3).

**Specificity/Sensitivity:** Each antibody in the Vav Isoform Antibody Sampler Kit detects endogenous levels of the specified Vav isoform.

**Source/Purification:** Polyclonal antibody is produced by immunizing animals with a synthetic peptide corresponding to the central sequence of human Vav3. Antibodies are purified by protein A and peptide affinity chromatography. Monoclonal antibodies are produced by immunizing animals with a synthetic peptide corresponding to residues in the carboxy terminus of Vav1 and near the carboxy terminus of human Vav2.

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

**Recommended Antibody Dilutions:**

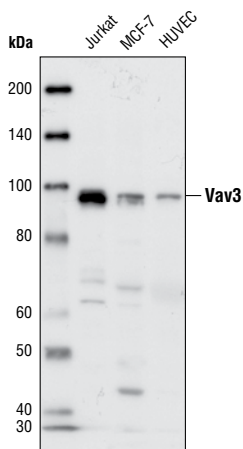
Western blotting 1:1000

Please visit [www.cellsignaling.com](http://www.cellsignaling.com) for a complete listing of recommended companion products.

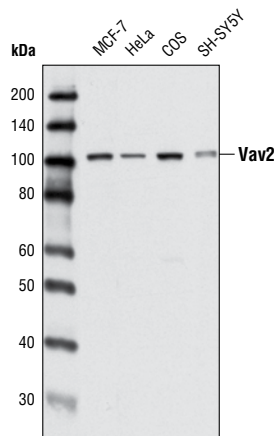
**Background References:**

- (1) Hornstein, I. et al. (2004) *Cell. Signal.* 16, 1–11.
- (2) Crespo, P. et al. (1997) *Nature* 385, 169–172.
- (3) Han, J. et al. (1997) *Mol. Cell. Biol.* 17, 1346–1353.

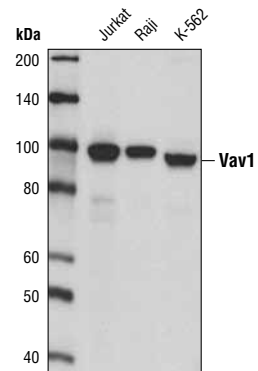
Rabbit monoclonal antibody is produced under license (granting certain rights including those under U. S. Patent No. 5,675,063) from Epitomics, Inc.



Western blot analysis of extracts from Jurkat, MCF-7 and HUVEC cells using **Vav3 Antibody #2398**.



Western blot analysis of extracts from various cell types using **Vav2 (C64H2) Rabbit mAb #2848**.



Western blot analysis of extracts from Jurkat, Raji and K-562 cells using **Vav1 (D45G3) Rabbit mAb #4657**.

## Western Immunoblotting Protocol (Primary Antibody Incubation in BSA)

For Western blots, incubate membrane with diluted antibody in 5% w/v BSA, 1X TBS, 0.1% Tween-20 at 4°C with gentle shaking, overnight.

### A Solutions and Reagents

**NOTE:** Prepare solutions with Milli-Q or equivalently purified water.

1. 1X Phosphate Buffered Saline (PBS)
2. **1X SDS Sample Buffer:** 62.5 mM Tris-HCl (pH 6.8 at 25°C), 2% w/v SDS, 10% glycerol, 50 mM DTT, 0.01% w/v bromophenol blue or phenol red
3. **Transfer Buffer:** 25 mM Tris base, 0.2 M glycine, 20% methanol (pH 8.5)
4. **10X Tris Buffered Saline (TBS):** To prepare 1 liter of 10X TBS: 24.2 g Tris base, 80 g NaCl; adjust pH to 7.6 with HCl (use at 1X).
5. Nonfat Dry Milk (weight to volume [w/v])
6. **Blocking Buffer:** 1X TBS, 0.1% Tween-20 with 5% w/v nonfat dry milk; for 150 ml, add 15 ml 10X TBS to 135 ml water, mix. Add 7.5 g nonfat dry milk and mix well. While stirring, add 0.15 ml Tween-20 (100%).
7. **Wash Buffer:** 1X TBS, 0.1% Tween-20 (TBS/T)
8. Bovine Serum Albumin (BSA)
9. **Primary Antibody Dilution Buffer:** 1X TBS, 0.1% Tween-20 with 5% BSA; for 20 ml, add 2 ml 10X TBS to 18 ml water, mix. Add 1.0 g BSA and mix well. While stirring, add 20 µl Tween-20 (100%).
10. **Phototope<sup>®</sup>-HRP Western Blot Detection System #7071:** Includes biotinylated protein ladder, secondary anti-rabbit (#7074) antibody conjugated to horseradish peroxidase (HRP), anti-biotin antibody conjugated to HRP, LumiGLO<sup>®</sup> chemiluminescent reagent and peroxide.
11. Prestained Protein Marker, Broad Range (Premixed Format) #7720
12. Biotinylated Protein Ladder Detection Pack #7727
13. **Blotting Membrane:** This protocol has been optimized for nitrocellulose membranes, which CST recommends. PVDF membranes may also be used.

### B Protein Blotting

A general protocol for sample preparation is described below.

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 per plate of 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 seconds to shear DNA and reduce sample viscosity.
5. Heat a 20 µl sample to 95–100°C for 5 minutes; cool on ice.
6. Microcentrifuge for 5 minutes.
7. Load 20 µl onto SDS-PAGE gel (10 cm x 10 cm).

**NOTE:** CST recommends loading prestained molecular weight markers (#7720, 10 µl/lane) to verify electrotransfer and biotinylated protein ladder (#7727, 10 µl/lane) to determine molecular weights.

8. Electrotransfer to nitrocellulose or PVDF membrane.

### C Membrane Blocking and Antibody Incubations

**NOTE:** Volumes are for 10 cm x 10 cm (100 cm<sup>2</sup>) of membrane; for different sized membranes, adjust volumes accordingly.

1. (Optional) After transfer, wash nitrocellulose membrane with 25 ml TBS for 5 minutes at room temperature.
2. Incubate membrane in 25 ml of blocking buffer for 1 hour at room temperature.
3. Wash three times for 5 minutes each with 15 ml of TBS/T.
4. Incubate membrane and primary antibody (at the appropriate dilution) in 10 ml primary antibody dilution buffer with gentle agitation overnight at 4°C.
5. Wash three times for 5 minutes each with 15 ml of TBS/T.
6. Incubate membrane with HRP-conjugated secondary antibody (1:2000) and HRP-conjugated anti-biotin antibody (1:1000) to detect biotinylated protein markers in 10 ml of blocking buffer with gentle agitation for 1 hour at room temperature.
7. Wash three times for 5 minutes each with 15 ml of TBS/T.

### D Detection of Proteins

1. Incubate membrane with 10 ml LumiGLO<sup>®</sup> (0.5 ml 20X LumiGLO<sup>®</sup>, 0.5 ml 20X Peroxide and 9.0 ml Milli-Q water) with gentle agitation for 1 minute at room temperature.

**NOTE:** LumiGLO<sup>®</sup> substrate can be further diluted if signal response is too fast.

2. Drain membrane of excess developing solution (do not let dry), wrap in plastic wrap and expose to x-ray film. An initial 10-second exposure should indicate the proper exposure time.

**NOTE:** Due to the kinetics of the detection reaction, signal is most intense immediately following LumiGLO<sup>®</sup> incubation and declines over the following 2 hours.