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**Technical Data Sheet**

**For research use only**
Not intended or approved for diagnostic or therapeutic use.

# Anti-hVps34 Antibody for Immunoprecipitation

Rabbit Polyclonal, Affinity Purified

**Catalog Number:** **Z-R015**

**Quantity:** 10 µg, 50 µg, or 250 µg

**Storage and Stability:** Store at -20°C. Product is stable for at least 6 months at -20°C from date of shipment.

**Antigen:** Human Vps34 (PIK3C3, Class III PI3-Kinase)

**Concentration:** 250 µg/mL

**Formulation:** Antibody is formulated in 100 mM Tris-HCl, 100 mM glycine, pH 7.5, and 50% glycerol.

**Application:** Non-inhibitory immunoprecipitation of human Vps34 from cell lysate. There is no need to elute the enzyme prior to assay. For Western Blot application, we recommend anti-hVps34 antibody, cat# Z-R016. See figure 1 and figure 2 below for data obtained using Z-R015.

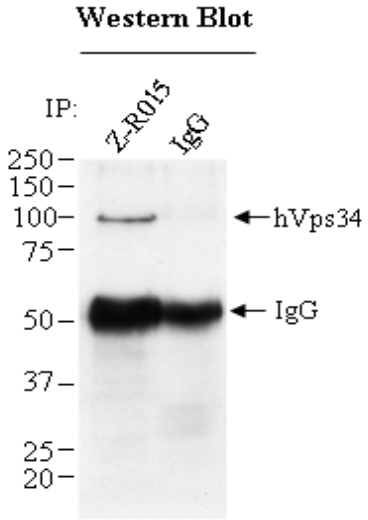


Fig.1. Lysates from HEK293T cells were immunoprecipitated with control rabbit IgG or Z-R015, then blotted with Z-R016.

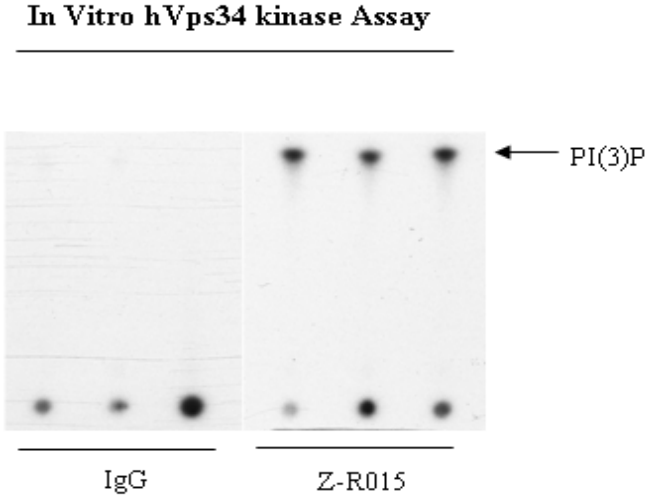


Fig. 2. Lysates from HEK-293T cells were immunoprecipitated with control rabbit IgG or Z-R015, then assayed for PI 3-kinase activity

**Reference:** Backer, J. M. (2008) The regulation and function of Class III PI3Ks: novel roles for Vps34. *Biochem. J.* **410**, 1–17

**Related Products:** Anti-hVps34 Antibody for Western Blot, cat# **Z-R016**
Class III PI3-K ELISA Kit, cat# **K-3000**
PI(3)P Mass ELISA, cat# **K-3300**
PI substrates for Vps34: cat#s **P-0016**, **P-0008**, **P-0008a**, and **P-0004**

# Protocol for Immunoprecipitation of hVps34 from Cells

1. Grow cells to 60-80% confluency in 10 cm dishes.
2. Induce quiescence by incubating overnight in medium containing 0.5% insulin-free BSA (starvation medium).
3. Cells are treated with hormone, stimulator, drug, or starvation medium, etc, and stopped by washing once with ice cold PBS (phosphate-buffered saline) and twice with Buffer A (20 mM Tris, pH 7.5, 137 mM NaCl, 1 mM $MgC1_2$, 1 mM $CaC1_2$, 100 mM NaF, 10 mM Na Pyrophosphate, and 100 µM $Na_3VO_4$).

Note: Untreated cells without serum starvation (step 2) and substance treatment (step 3) will yield hVps34 with high activity.

4. Remove solution and add 1 mL of ice cold Lysis Buffer (Buffer A containing 1% Nonidet P-40 (Sigma), 10% glycerol, and 0.35 mg/mL protease inhibitor PMSF (phenylmethylsulfonyl fluoride). Rock plates on ice for 20 minutes.
5. Scrape cells from dish, transfer to 1.5 mL micro centrifuge tubes. Centrifuge for 10 minutes at 13,000 g to sediment insoluble material.
7. Transfer supernatant to new tubes, add 1-2 µg of anti-hVps34 IP antibody (cat # Z-R015) and incubate for at least 4 hours (can be extended over night) at 4°C.
8. Add 60 µL of 50% slurry of Protein-A Sepharose in PBS to each tube and incubate for 1 hour at 4°C.
9. Collect immunoprecipitated enzyme by quick centrifuging and discard supernatant. Wash immunocomplexes with freshly prepared buffers in following order:
    - Three times with PBS/1% Nonidet P-40
    - Three times with 100 mM Tris-HCl, pH 7.5/500 mM LiCl.
    - Two times with TNE (10 mM Tris-HCl, pH 7.5, 100 mM NaCl, and 1 mM EDTA).
    - Two times with Vps34 kinase reaction buffer (User's choice. Two recipes are provided below)

    Recipe #1: 50 mM HEPES pH 7.5, 150 mM NaCl, 1 mM CHAPS, 5 mM $MnCl_2$, 1 mM DTT and 50 µM ATP.
    Recipe #2: 10 mM Tris pH 8, 100 mM NaCl, 1 mM EDTA, 10 mM $MnCl_2$, and 50 µM ATP.

    Aspirate last wash as completely as possible, and proceed with kinase reactions or other applications immediately.

Note: We recommend using immunoprecipitated hVps34 enzyme immediately for activity assays. Sepharose beads should not be frozen at any time.