Echelon Biosciences Inc.
675 Arapeen Drive, Suite 302
Salt Lake City, UT 84108
Telephone 866-588-0455
Fax 801-588-0497
echelon@echelon-inc.com
www.echelon-inc.com

Technical Data Sheet

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

Anti-hVps34 Antibody for Western Blot

Rabbit Polyclonal, Purified

Catalog Number: **Z-R016**

Quantity: 10 µg, 50 µg, and 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: Residues 407-420 of human hVps34 (PIK3C3, Class III PI3-Kinase)

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

Application: As primary antibody for Western Blot at 2.5 µg/mL concentration. Z-R016 works well with immunoprecipitated hVps34 (Fig.1) or partially purified hVps34 (Fig. 2, left panel). It also works with whole cell lysate (Fig.2 right panel). For IP application, we recommend using Anti-hVps34 antibody, cat # Z-R015.

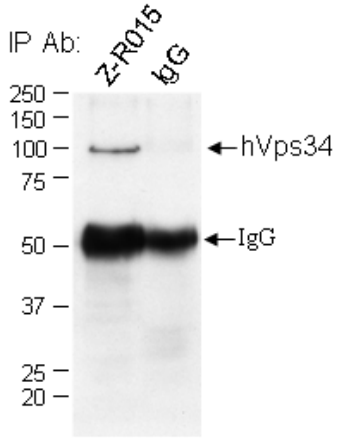

Fig.1. Lysates from HEK293T cells were immunoprecipitated with control rabbit IgG or Z-R015, then blotted with Z-R016.

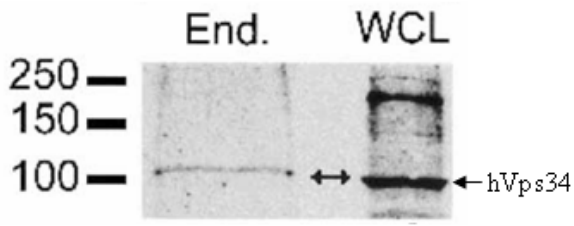

Fig. 2 Purified endosomal fractions (left hand panel) or whole cell lysates (WCL) from HeLa cells (right hand panel) were blotted with Z-R016. (from Murray et al., Traffic 3:416-427 2002)

References:

1. Murray, J. T., Panaretou, C., Stenmark, H., Miaczynska, M. and Backer, J. M. (2002) Role of Rab5 in the recruitment of hVps34/p150 to the early endosome. *Traffic* **3**, 416–427
2. 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 Immunoprecipitation (IP), cat# **Z-R015**
Class III PI3-K ELISA Kit, cat # **K-3000**
PI(3)P Mass ELISA Kit, cat # **K-3300**

Z-R016 Western Blot Protocol

1. Perform SDS-polyacrylamide gel electrophoresis (SDS-PAGE) and transfer to membrane (electroblotting).
2. Wash the membrane twice with distilled water. If desired, stain the membrane with Ponceau Red solution for 5 minutes to visualize protein bands. (Stock solution: 2% Ponceau S in 30% trichloroacetic acid and 30% sulfosalicylic acid; dilute 1:10 for use.) Rinse the membrane in water until protein bands are distinct and mark the position of the molecular weight markers with a ballpoint pen or pencil. The Ponceau Red stain will be washed off the membrane during the blocking step. Note: Do not let the blot dry out at any step through development, as this will cause an increase in background.
3. Block the blotted membrane in freshly prepared blocking buffer (25 mM Tris-HCl, pH 7.4, 137 mM NaCl (TBS), 0.01%Tween-20, 3% nonfat dry milk) for 30–60 minutes at room temperature or over night at 4 °C with constant agitation.
4. Dilute the anti-hVps34 antibody for WB (Z-R016) to 2.5 µg/mL in fresh blocking buffer. Incubate the membrane in the anti-hVps34 antibody solution for 2 hours at room temperature or overnight at 4 °C with agitation.
5. Wash the membrane three times for 5 minutes each with wash buffer (TBS- 0.01%Tween-20).
6. Incubate the membrane with a goat anti-rabbit HRP-conjugated antibody (user's choice) diluted in fresh blocking buffer for 30-60 minutes at room temperature with agitation.
7. Wash the membrane three times for 5 minutes each time with wash buffer.
8. Detect hVps34 using 1- 2 mL of TMB precipitating substrate (cat# K-TMBP). Other detection system of choice can be used as well, e.g. enhanced chemiluminescence (ECL), following instructions provided by the supplier.