

Anti-Verotoxin (E. coli) / Shiga Toxin (S. dysenteriae) antibody, rabbit serum

64-025 100 μl

Shipping and Storage: Shipped at 4° C or -20° C. Store at -20° C.

Immunogen: Initial immunization by VT1 toxoid and boostered by VT1 toxin.

Form: Rabbit antiserum added with 0.09% sodium azide.

Reactivity: VT1 and VT2 of *E. coli* VTEC strain and Shiga toxin of *Shigella dysenteriae*. Applications:

- 1) Western blotting (2,000 fold dilution) 2) Immunoprecipitation
- 3) ELISA Other applications have not been tested.

Background: Vero toxins, VT1 and VT2 are produced by Vero toxin producing *E.coli* (VTEC) or Entrohaemorrhagic *E. coli* (EHEC) and have lethal activity to Vero cells. The primary structure of VT1 is identical or nearly identical to Shiga toxin (Stx) produced by *Shigella dysenteriae* serotype 1 and also called Slt 1 (Shiga-like toxin 1). VT is composed from one A subunit and five B subunits. Some *E. coli* strains produce both VT1 and VT2, and they share sequence identity of 55 %.

Data link: GenBank M16625 Shiga-like toxin I subunit A and subunit B UniProtKB/Swiss-Prot Q9FBI2 Shiga toxin subunit A UniProtKB/Swiss-ProtQ7BQ98 Shiga toxin subunit B

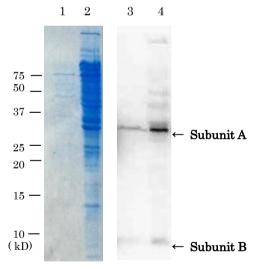


Fig.1.Detection of VT1 by western blotting

SDS-PAGE of culture medium (50 $\,$ μ l) of VTEC,

- 1. SDS-PAGE of crude extracts of VTEC cells (20 μ g),
- 2. Western blotting of culture medium of VTEC
- 3. Western blotting of crude extracts of VTEC cells.

The anti-Verotoxin antibody was used at 1/2,000 dilution. As the secondary antibody, HRP conjugated goat anti-rabbit IgG was used at 1/20,00 dilution

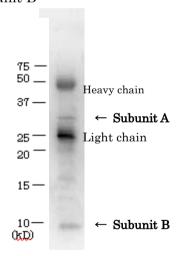


Fig.2. Immunoprecipitation of VT1 from culture medium of VTEC with anti-Verotoxin antibody.

The antibody for immunoprecipitation was used at 1/200 dilution.

Arrows shows subunit A and subunit B of VT1. Heavy chain and Light chain indicate those of IgG.



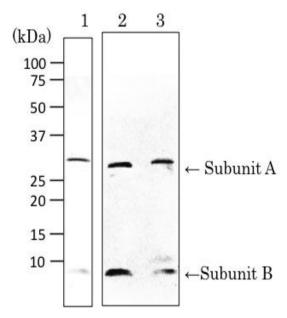


Fig.3. Detection of VT1 and VT2 by Western blotting with ant-Vero toxin.

- 1. Culture medium of *E. coli* O157:H7 (100 μ l)
- 2. Purified VT1 (50 ng)
- 3. Purified VT2 (50 ng)

The anti-Verotoxin antibody was used at 1/2,000 dilution and As the secondary antibody, HRP conjugated goat anti-rabbit IgG was used at 1/20,000 dilution

Arrow shows subunit A and subunit B.

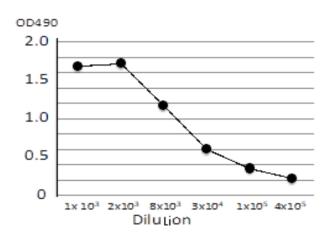


Fig.4. Titration of antibody reactivity of anti-Vero Toxin by indirect ELISA using crude extract of *E.coli* O157:H7

The wells of plate were coated with crude extract of O157:H7 (100 μ l, 1 μ g/ml). After blocking with 5% skim milk, 100 μ l of antibody at the indicated dilution was added to the each well. HRP-conjugate goat anti-mouse IgG (100 μ l, x2000 dilution) was added. Color was developed with orthophenylenediamine as substrate. Optical densities (OD) measured at 490nm.