

Anti-dVPE antibody, rabbit polyclonal

81-118 100 µg

Storage: Ship at 4°C and store at -20°C. Do not freeze below -20°C.

Reactivity: *Arabidopsis thaliana*. Not tested in other species.

Immunogen: Recombinant His6-dVPE (amino acids 40-407) of *Arabidopsis thaliana*.

Applications:

1. Western blotting (1/5,000)
2. Immunofluorescent staining (1/500)
3. Immunoelectron microscopy (1/5,000)

Purity: IgG fraction purified with protein A from the rabbit antiserum to dVPE.

Form: 2 mg/ml in PBS, 50% glycerol. Filter-sterilized. No preservative or carrier protein

Background: δ VPE (Vacuolar-Processing Enzyme delta-isozyme) is Asparagine specific endopeptidase that may be involved in processing of proteins targeted to vacuoles (By similarity). Probably involved in post-translational proteolysis of seed storage proteins in the protein storage vacuole of developing seeds (PubMed:[12417707](#), PubMed:[14688293](#)). Exhibits a caspase-1-like activity in extracellular granules. At the early stage of seed development, required for the formation of the seed coat, by regulating cell death of specific cell layers in inner integument (PubMed:[15705955](#)). Length: 466 amino acids. Mass: 52,085. Signal peptide of 24 amino acids is removed in mature protein.

Subcellular localization: Seed specific. Restricted to developing seeds at 7 days after anthesis, and, at lower levels, detected in flowers siliques, specifically in seed coats

Data Link: UniProtKB [Q9LJX8](#) (VPED_ARATH)

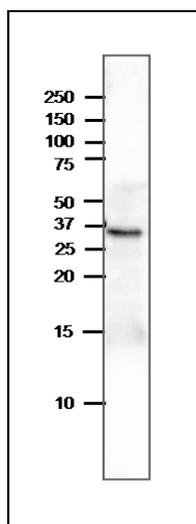


Fig.1 Western blot of dVPE in extract of maturing siliques of *Arabidopsis thaliana*

Crude extract of maturing siliques of *Arabidopsis thaliana* was run on SDS-PAGE (15-20% gradient gel) and blotted to PVDF membrane by wet system. Blocking was done with 3% skim milk. The anti-PYK10 (intermial) antibody was used at 0.4 µg/ml. Secondary antibody (goat anti-rabbit IgG antibody HRP-conjugated, ab97051) was used at 1/10,000 dilution.

dVPE is produced as an inactive precursor with 52 kDa mass and processed into mature active form with 37 -38 kDa mass (Ref 1)

DIC

anti-dVPE antibody

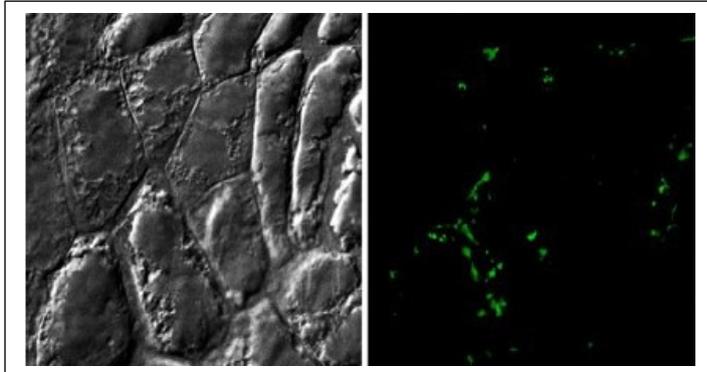


Fig 2 Immunofluorescence analysis of surfaces of the inner integuments with anti-dVPE antibodies

Left: Differential Interference Contrast image.

Right: Immunofluorescent staining.

The developing seeds of *Arabidopsis* that had walking-stick-shaped embryos were fixed for 40 min in 7.2% (w/v) formaldehyde, 0.1% (v/v) Nonidet P-40, 10% (v/v) dimethyl sulfoxide, and 50 mM Na-phosphate buffer, pH 7.2, washed twice with TBS-T for 5 min, incubated in TBS-T containing 5% (w/v) Cellulase Onozuka R-10 and 2% (w/v) Pectolyase Y-23 for 20 min at 30°C, washed twice with TBS-T, incubated in blocking buffer (2% BSA and TBS-T) for 30 min, incubated with anti-dVPE antibodies (diluted 500-fold) in the blocking buffer for 40 min, washed three times for 5 min each, incubated for 1 h with goat anti-rabbit IgG antibodies conjugated with Alexa Fluor 488, washed three times for 5 min with TBS-T, and mounted

Reference. This antibody was described in Ref.1 and used in the following publications.

1. Nakaune S et al. A vacuolar processing enzyme, deltaVPE, is involved in seed coat formation at the early stage of seed development. *Plant Cell*. 2005 Mar;17(3):876-87. PMID: [15705955](#). **WB, IF, Immunoelectron microscopy (arabidopsis)**
2. Kunieda T et al. NAC family proteins NARS1/NAC2 and NARS2/NAM in the outer integument regulate embryogenesis in *Arabidopsis*. *Plant Cell*. 2008 Oct;20(10):2631-42. PMID: [18849494](#) **WB (arabidopsis)**
3. Kunieda T et al. Spatiotemporal secretion of PEROXIDASE36 is required for seed coat mucilage extrusion in *Arabidopsis*. *Plant Cell*. 2013 Apr;25(4):1355-67. PMID: [23572548](#). **WB (arabidopsis)**