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Product no AS13 2757

LEA4-25 | Group 4-late embryogenesis abundant protein

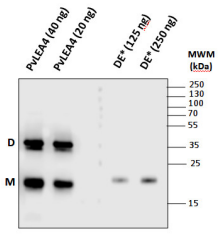
Product information

Immunogen	Recombinant *Phaseolus vulgaris* PvLEA 4-1 sequence derived from Pv01G142000
Host	Rabbit
Clonality	Polyclonal
Purity	Serum
Format	Lyophilized
Quantity	50 µl
Reconstitution	For reconstitution add 50 µl of sterile water
Storage	Store lyophilized/reconstituted at -20°C; once reconstituted make aliquots to avoid repeated freeze-thaw cycles. Please remember to spin the tubes briefly prior to opening them to avoid any losses that might occur from material adhering to the cap or sides of the tube.
Additional information	This antibody does not recognize group 4 LEA proteins from *Arabidopsis thaliana*.

Application information

Recommended dilution	1 : 2000 (WB)
Expected | apparent MW	16 | 18 kDa
Confirmed reactivity	*Phaseolus vulgaris*
Predicted reactivity	*Glycine tomentosa* Species of your interest not listed? Contact us
Not reactive in	*Arabidopsis thaliana*
Additional information	The PvLEA4-1 protein from common bean presents a deduced molecular mass of 16 kDa; however, as other LEA and disordered proteins it migrates with an apparent higher molecular mass, possibly due to post-translational modifications

application information



Phaseolus vulgaris seed protein extracts which is an extract enriched in hyrdophilic proteins, obtained in a following way: Dry seeds were used to separate embryos from cotyledons. Approximately 500 mg of embryos were ground in a mortar in the presence of liquid nitrogen or dry ice. The powder was resuspended and homogenized in 1 mL of 20 mM TES pH 8.0, 0.5 M NaCl, 10 mM Na2VO3, 10 mM NaF, 1 mM PMSF and one tablet/10 mL (protease inhibitor cocktail tablet, Roche). The suspension was centrifuged at 14,000 rpm in a microcentrifuge during 10 min at 4ºC, supernatant was separated and centrifugation was repeated once more. Then, supernatant was boiled 10 min and transfer to ice for 15 min, the suspension was centrifuged at 14,000 rpm in a microcentrifuge during 10 min at 4ºC, and the

boil-ice-centrifugation procedure was repeated twice. Subsequently, the supernatant was dialyzed against 10 mM phosphate buffer pH 8.0 in the cold room. Concentration of the protein extract was determined by standard procedures and its integrity was verified by SDS-PAGE.
Following samples were separated: PvLEA4 is a recombinant PvLEA4-1 proein which does not contain any tag and was purified from bacterial extract. M: monomoer, D: dimer. DE*: total protein extract from dry *Phaseolus vulgaris* embryos. Proteins were separated on 12% SDS-PAGE and blotted 1h to nitrocellulose membrane using semi-dry transfer for 1 h. Blots were blocked ON at 4ºC in 2% non-fat milk with agitation. Blots were incubated in the primary antibody at a dilution of 1: 1 000 for 1h at RT with agitation. The antibody solution was decanted and the blot was rinsed briefly twice, then washed once for 15 min and 3 times for 5 min in TBS-T at RT with agitation. Blot was incubated in secondary antibody (anti-rabbit IgG horse radish peroxidase conjugated, from Agrisera, AS09 602) diluted to 1:25 000 in 2% non-fat milk for 1h at RT with agitation. The blot was washed as above and developed for 5 min with ECL according to the manufacturer's instructions. Exposure time was 1 min.

Courtesy of Dr. Alejandra A. Covarrubias, UNAM, Mexico