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Product no AS13 2758A

Anti-LEA4-5 | Late embryogenesis abundant protein 4-5 (affinity purified)

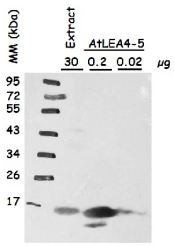
Product information

Immunogen	recombinant LEA4-5 from *Arabidopsis thaliana:* UniProt: Q9FG31, TAIR: AT5G06760, a group 4 LEA protein (Battaglia et al., 2008)
Host	Rabbit
Clonality	Polyclonal
Purity	Immunogen affinity purified serum in PBS pH 7.4.
Format	Lyophilized
Quantity	50 µg
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.

Application information

Recommended dilution	1 : 1000 (WB)
Expected | apparent MW	16 kDa
Confirmed reactivity	*Arabidopsis thaliana*
Predicted reactivity	*Arachis hypogaea*, *Brassica* sp., *Glycine max*, *Medicago truncatula*, *Phaseolus vulgaris*, *Thellungiella halophila* Species of your interest not listed? Contact us
Not reactive in	No confirmed exceptions from predicted reactivity are currently known

Application information



Protein extracts were obtained from Arabidopsis thaliana seeds (extract), beside recombinant AtLEA4-5 loaded in indicated amounts. Following extraction buffer was used: 0.7 M sucrose, 0.5 M Tris-base, 30 mM HCl, 50 mM EDTA, 0.1 M KCl, 2% -mercaptoethanol, 12 mg/ml poly-vinyl-poly-pyrrolidone (PVPP). This buffer was complemented with 2mL of equilbrated phenol before extraction. Samples were centrifuged and the protein phase was recovered; the extracted proteins were precipitated with 0.1 m ammonium acetate dissolved in methanol, centrifuged and the pellet washed with cold (-20°c) 80% acetone. The protein pellet was dissolved in SDS-solubilization buffer (1% CHES, 2% SDS, 2% ß-mercaptoethanol, 10% glycerol). Thirty µg of seed protein extract was denatured with Laemmli buffer at 95 °C for 5 min and proteins were separated on 15% SDS-PAGE and blotted 1.5 h to

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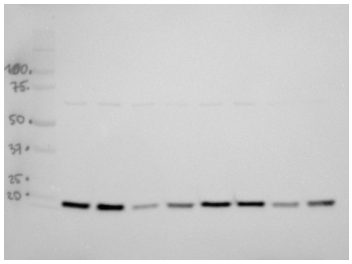
nitrocellulose membrane in a liquid transfer system. Blots were blocked with 2% skim milk ON at 4°C with agitation. After rinsing with TBS, blots were incubated in the primary antibody at a dilution of 1:1 000 (anti-AtLEA4-5) for 3h at 4°C with agitation. The antibody solution was decanted and the blot was rinsed briefly, then washed once for 15 min and 3 times for 5 min in TBS-T at 4°C with agitation. Then, blots were incubated in secondary antibody (anti-rabbit IgG horse-radish peroxidase conjugate) diluted to 1:25 000 in for 1h at RT with agitation. The blot was washed as above and developed for 2 min with chemiluminescent detection reagent. Exposure time was for 30 seconds.

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



30 µg of total seed protein isolated from *Arabidopsis thaliana* dry seeds and seeds germinating for 24h, extracted with a buffer containing urea, thiourea, CHAPS and Triton X-100 (Heidorn-Czarna et al., 2018) were denaturated with Laemmli buffer at 95°C for 5 min and separated on 12% SDS-PAGE. Afterwards the gel was blotted for 30 min to PVDF membrane using semi-dry transfer (Trans-Blot Turbo, Bio-Rad). The efficiency of transfer was confirmed by staining of the membrane with Coomassie solution. Blot was blocked with 5% milk in TBS-T for 1h at RT with agitation. Blot was incubated in the primary antibody (anti-LEA4-5, AS13 2758A) at a dilution 1:1000 in 5% milk in TBS-T for 1.5h /RT with agitation. The antibody solution was decanted and the blot was rinsed briefly twice, then washed once for 15 min and 2 times for min in TBS-T at RT with agitation. Blot was incubated in Agrisera secondary antibody (goat anti-rabbit IgG, HRP-conjugated, AS09 602) diluted to 1:20 000 in 5% milk in TBS-T for 1h/RT with agitation. The blot was washed as above and developed with chemiluminescent detection reagent, using GBox imager (Syngene). Exposure time was: 3 minutes.

Analyzed samples:

1. Wild-type, **dry seeds**, I biological replicate

2. *mutan*t, **dry seeds**, I biological replicate
3. Wild-type **germinating for 24h**, I biological replicate
4. *mutant* **germinating for 24h**, I biological replicate
5. Wild-type, **dry seeds**, II biological replicate
6. *mutan*t, **dry seeds**, II biological replicate
7. Wild-type **germinating for 24h**, II biological replicate
8. *mutant* **germinating for 24h**, II biological replicate

Courtesy Dr Małgorzata Heidorn-Czarna, University of Wrocław, Poland