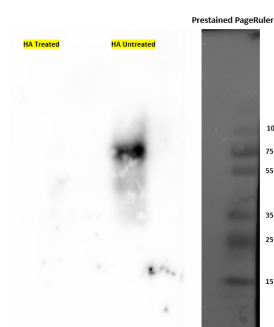


Product no **AS18 4176****Anti-HA (monoclonal, Clone HA,C5)****Product information**

Immunogen	KLH-conjugated synthetic peptide: YPYDVPDYA
Host	Mouse
Clonality	Monoclonal
Purity	Immunogen affinity purified in PBS, pH 7.4,. Contains 0.05% sodium azide.
Format	Liquid
Quantity	100 µg
Storage	Store at -20 °C, Store in small 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** 1:200 (IP, IL), 1:1000 (WB)**Confirmed reactivity** HA-tagged proteins in transfected mammalian cells or expressed under native promoter**Application example**

Tagged protein is expressed under native promoter and so IP is performed prior to Western Blotting. Starting material of 200mg of root tissue was used for membrane protein extraction with buffer components Tris pH7.5, Tris pH 6.8, D-Sorbitol, casein, Na EDTA, Na EGTA, NaF, Na molybdate, -glycerophosphate, KH₂PO₄ 7.8 and Protease inhibitor cocktail and denatured with 1x Lameli buffer at 85 °C for 10 min were separated on 10 % SDS-PAGE and blotted 1.5h to PVDF using wet transfer. Blot was blocked with 5% milk for 2h/RT with agitation. Blot was incubated in the primary antibody at a dilution of 1: 5 000 (both anti-GFP & anti-HA) for ON/4 °C with agitation in TBS-T. 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 Agrisera matching secondary antibody (anti-mice HRP, anti-Chicken HRP) diluted to 1:10 000, 1:5000, in for 2h/RT with agitation. The blot was washed as above and developed with chemiluminescence detection reagent. Exposure time was 2mins (anti-GFP) and 2sec (anti-HA).

Courtesy of Stanislava Sládečková, Developmental and Cell Biology of Plants, Masaryk University, CEITEC, Czech Republic