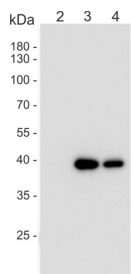


Product no **AS15 3036****Anti-DYKDDDDK (binds to Sigma FLAG®) (mouse antibody, monoclonal, Clone 1E6)****Product information**

Immunogen	KLH-conjugated synthetic peptide: DYKDDDDK (Sigma FLAG®)
Host	Mouse
Clonality	Monoclonal
Purity	Immunoglobulin Protein A/G purified in PBS, pH 7.4. Contains 0.055% sodium azide.
Format	Liquid
Quantity	100 µg
Storage	Store at -20 °C for up to 1 year, 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 : 100 - 1000 (WB) 1 : 20 (IP), 1 : 20 (IHC)
Confirmed reactivity	DYKDDDDK epitope tag (Sigma FLAG®), fused to proteins in mammalian cells
Not reactive in	No confirmed exceptions from predicted reactivity are currently known
Selected references	<u>Durai</u> et al. (2020). Increased ethylene production by overexpressing phosphoenolpyruvate carboxylase in the cyanobacterium <i>Synechocystis</i> PCC 6803. <i>Biotechnol Biofuels</i> . 2020 Jan 28;13:16. doi: 10.1186/s13068-020-1653-y. <u>Wardhan</u> et al. (2017). Chickpea transcription factor CaTLP1 interacts with protein kinases, modulates ROS accumulation and promotes ABA-mediated stomatal closure. <i>Sci Rep</i> . 2016 Dec 9;6:38121. doi: 10.1038/srep38121.



1 and 2. pBA002 vector as negative control (technical replicates).
3 and 4. pBA002:AtACO4 (technical replicates)

18 µg/well of total protein extracted freshly from *Nicotiana benthamiana* with following buffer components: Tris-HCl pH 7.5 100 mM, Urea 8 M, Triton x-100 (0.2%), Sarkosyl (0.2%), PMSF 1 mM, Pepstatin 1 µg/mL, Leupeptin (1 µg/mL), NEM 2 mM and Iodoacetamida 10 mM and denatured with 4x Laemmli Sample Buffer at 95 °C for 5 min. Protein samples were separated on 12% SDS-PAGE and blotted for 1h to PVDF (pore size of 0.2 µm), using semi-dry transfer. Blot was blocked with 3% milk in TBS-T for 1h/RT with agitation. Blot was incubated in the primary antibody at a dilution of 1 : 1000 (anti-ACO4) or 1:2000 (anti-flag, Agrisera, No. AS15 3036, Lot. 1902) in 3% milk in TBS-T ON/4 °C with agitation (anti-ACO4 and anti-FLAG in corresponding membrane). The antibody solution was removed and the blot was washed three times for 10 min in TBS-T at RT with agitation. Blot was incubated with Peroxidase labelled anti-mouse antibody diluted to 1:25 000 for 1h/RT with agitation. The blot was washed as above and developed for 30 min with AgriseraECLSuperBright. Exposure time was 60 seconds. Asterisk denotes a band recognized in all samples.

Courtesy of Dr. Maria Lois, CRAG, Spain