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Product no AS20 4440

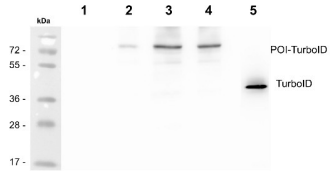
BirA (mutated/TurboID)

Product information

Immunogen	Recombinant mutated BirA protein from *E.coli* produced using the following plasmid: TurboID-His6_pET21a, (Plasmid #107177). Expression was done in a vector that allowed for the generation of an untagged protein (without HIS6tag).
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 : 5000 (WB)
Expected | apparent MW	35 kDa
Confirmed reactivity	BirA (mutated/TurboID)
Predicted reactivity	mini TurboID
Not reactive in	No confirmed exceptions from predicted reactivity are currently known
Selected references	Medica et al. (2023). Proximity-dependent mapping of the HCMV US28 interactome identifies RhoGEF signaling as a requirement for efficient viral reactivation. PLoS Pathog. 2023 Oct 2;19(10):e1011682.doi: 10.1371/journal.ppat.1011682. Shi et al. (2023). Protocol to identify protein-protein interaction networks in Solanum tuberosum using transient TurboID-based proximity labeling. STAR Protoc. 2023 Sep 20;4(4):102577.doi: 10.1016/j.xpro.2023.102577.



1 – 20 µg *A. thaliana* – Wt Col-0 (Negative control)
2 – 20 µg of *A. thaliana* expressing POI-TurboID fusion (Independent line 1)
3 – 20 µg of *A. thaliana* expressing POI-TurboID fusion (Independent line 2)
4 – 20 µg of *A. thaliana* expressing POI-TurboID fusion (Independent line 3)
5 – 10 ng of purified TurboID (Positive control)

20 µg/well of total protein were extracted from *Arabidopsis thaliana* leaf material in diluted HENS (25mM HEPES pH 7.7, 1mM EDTA, 2.5 % SDS) and stored at -80°C. Samples were denatured in 1x protein loading dye (0.5% Sodium dodecyl Sulfate, 0.002% Bromophenol Blue, 10% glycerol, and 50 mM Tris-HCl pH6.8) at 95°C for 5 min. Samples were separated on 4-16% gradient SDS-PAGE gel and blotted 1h to a nitrocellulose membrane (pore size of 0.45 um), using a semi-dry transfer. Blot was blocked with 5% milk in TBS-T at 4°C/ON without agitation. Blot was incubated in the primary antibody at a dilution of 1:5000 in 5% milk in TBS-T, at 4°C, ON without agitation. The antibody solution was decanted and the blot was rinsed briefly, then washed three times for 10 min in TBS-T at RT with agitation. Blot was incubated in Agrisera matching secondary antibody (anti-rabbit IgG horse radish peroxidase conjugated, AS09 602) diluted to 1:25 000 in TBS-T for 1h at RT with agitation. The blot was washed as above and developed for 2 min with Agrisera ECLBright. Exposure time was 172 seconds.

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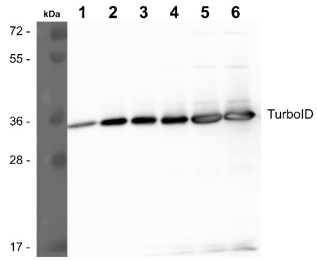

1 – 1 ng of purified TurboID 2 – 5 ng of purified TurboID 3 – 10 ng of purified TurboID 4 – 25 ng of purified TurboID 5 – 50 ng of purified TurboID 6 – 75 ng of purified TurboID Mark: PageRulerTM Plus Prestained Protein Ladder; ThermoFisher Scientific; MW of TurboID = 35 kDa

1ng, 5 ng, 10 ng, 25 ng, 50 ng, and 75 ng loaded into wells 1, 2, 3, 4, 5, and 6, respectively, of purified TurboID in 1x Phosphate Buffer Saline (PBS) were stored at -80°C. Samples were denatured in 1x protein loading dye (0.5% Sodium dodecyl Sulfate, 0.002% Bromophenol Blue, 10% glycerol, and 50 mM Tris-HCl pH6.8) at 95°C for 5 min. Samples were separated on 4-16% gradient SDS-PAGE gel and blotted 1h to a nitrocellulose membrane (pore size of 0.45 um), using a semi-dry transfer. Blot was blocked with 5% milk in TBS-T at 4°C/ON without agitation. Blot was incubated in the primary antibody at a dilution of 1:1000 in 5% TBS-T, at 4°C, ON without agitation. The antibody solution was decanted and the blot was rinsed briefly, then washed three times for 10 min in TBS-T at RT with agitation. Blot was incubated in Agrisera matching secondary antibody (anti-rabbit IgG horse radish peroxidase conjugated, AS09 602) diluted to 1:25 000 in TBS-T for 1h at RT with agitation. The blot was washed as above and developed for 2 min with Agrisera ECLBright. Exposure time was 92 seconds.

Courtesy of Eli Gordon and Dr. Patrick Treffon, Elizabeth Vierling Lab Department of Biochemistry and Molecular Biology University of Massachusetts Amherst, USA