

This product is for research use only (not for diagnostic or therapeutic use)

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## Product no AS20 4440

## BirA (mutated/TurbolD)

## **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

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

**Confirmed reactivity** BirA (mutated/TurboID)

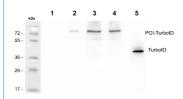
Predicted reactivity mini TurboID

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)

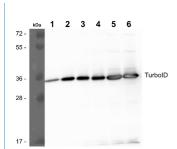
20 μg/well of total protein were extracted from Arabidopsis thaliana leaf material in diluted HENS (25mM HEPES pH 7.7, 1mM EDTA, 2.5 % SDS) and stored at -80°C. Samples were denatured in 1x protein loading dye (0.5% Sodium dodecyl Sulfate, 0.002% Bromophenol Blue, 10% glycerol, and 50 mM Tris-HCl pH6.8) 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 TurbolD 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, <u>AS09 602</u>) 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 <u>Agrisera ECLBright</u>. 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