

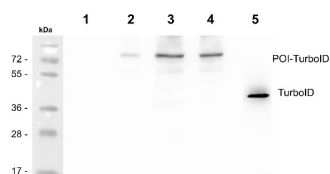
Product no **AS20 4440**
BirA (mutated/TurboID)

Product information

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|-----------------------|---|
| Immunogen | Recombinant mutated BirA protein from <i>E.coli</i> 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. |

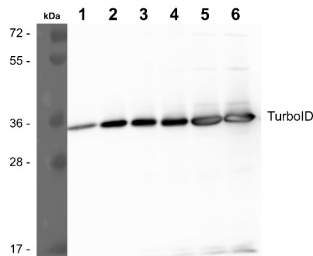
Application information

| | |
|-------------------------------|---|
| 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 | To be added when available, antibody available in January 2021. |



- 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 µm), 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.



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: PageRuler™ 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