**Product Information**

**Background**
Iron-hydrogenase HydA2 is catalyzing reversible oxidation of molecular hydrogen. In *Chlamydomonas* the protein is present in low levels of 1 µg/liter of culture. Synonyme: HYD1/1

**Immunogen**
Recombinant, full length *Chlamydomonas reinhardtii* HydA-2 Q8VZZ0

**Host**
Rabbit

**Clonality**
Polyclonal

**Purity**
Serum

**Format**
Lyophilized

**Quantity**
100 µl

**Reconstitution**
For reconstitution add 100 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 tubes briefly prior to opening them to avoid any losses that might occur from lyophilized material adhering to the cap or sides of the tubes.

**Tested Applications**
Western blot (WB)

**Related Products**
- AS09 600 | anti-HydA | Iron-hydrogenase HydA2 rabbit antibodies
- Collection of antibodies to *Chlamydomonas reinhardtii*
- Algal protein extraction buffer
- Secondary antibodies

**Additional Information**
In *Chlamydomonas* HydA is present in low levels of 1 µg/liter of culture. Therefore, an induction of cells by anaerobic adaptation or sulfur depravation (10 x higher amount than with anaerobic adaptation) is necessary for successful detection using this antibody. Methods of HydA induction are described in Hemschemeier et al. 2009.

To detect HydA1/2 in *Chlamydomonas* extracts amount loaded per well corresponds to 2 µg of chlorophyll for sulfur deprived cells, where relatively much HydA1 is synthesized or corresponds to 2-4 µg of artificially anaerobic induced cultures, where the HydA1 protein level is lower. This antibody is recognizing 1 ng of recombinant HydA protein.

**Application Information**

**Recommended Dilution**
1 : 5000 (WB)

**Expected | apparent MW**
53.7 | 48 kDa (after transit peptide is cleaved)

**Confirmed Reactivity**
*Chlamydomonas reinhardtii*

**Predicted Reactivity**
*Ostreococcus* sp.

**Not Reactive in**
No confirmed exceptions from predicted reactivity are currently known.

**Additional Information**
HydA1 (497aa) has a calculated MW of 53.1 kDa, but this is including the signal peptide, which gets cleaved off. The protein without TP has a calculated MW of 47.5 kDa and runs according to its size at about 48 kDa.

HydA2 (505aa) has a calculated MW of 53.7 kDa, but this is including the signal peptide, which gets cleaved off. The protein without TP can only be estimated, since the cleavage site is known only from in silico analysis. It has a calculated MW of 47.3 kDa and should run in the gel also according to its size.

This antibody is binding recombinant HydA1/2 protein.
Selected references

Weiner et al. (2018). Overcoming the expression barrier of the ferredoxin hydrogenase chimera in Chlamydomonas reinhardtii supports a linear increment in photosynthetic hydrogen output. Algal Research Volume 33, July 2018, Pages 310-315


Application example

50ng of purified protein (HydA1 and HydA2) were separated on 10% SDS-PAGE and blotted 25 min to PVDF membrane. Filters were blocked 1h with 3% low-fat milk powder in PBS-T (0.1% TWEEN 20) and probed with anti-HydA1/2 (AS09 514, 1:5000, over night at 4°C) and secondary anti-rabbit (1:10 000, 1 h) antibody (HRP conjugated, manufacture Pierce) in PBS-T containing 3% low fat milk powder. Antibody incubations were followed by washings in PBS-T (10, +10min and PBS (+5, +5 min). All washing steps were performed at RT with agitation. Signal was detected with ECL (Millipore) using CCD camer. Exposure time was 20 min.

The heterolog expressed proteins have both calculated MWs of 51 kDa (due to the tag) and run according to their size.

Courtesy Dr. Thomas Happe