iBodies
synthetic antibodies

iBodies Technology

iBodies anti-polyHis 488 and anti-polyHis 800 were specially developed to target the most commonly used affinity tag, the polyhistidine-tag (His-tag).

Anti-polyHis iBodies bind the polyhistidine tag via an interaction with cobalt-charged tris-nitrilotriacetic acid (tris-NTA (Co^{2+})), where tris-NTA functions as a chelating agent that stably binds the metal ion, which in turn forms a strong bond with the polyhistidine-tag.

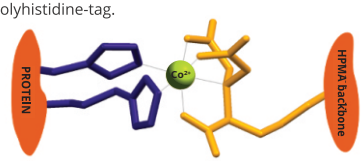

Figure 1: Illustrative representation of tris-NTA (Co^{2+}) interaction with polyhistidine-tag

Product Description

Anti-polyHis 488:
~ M_r = 99 kDa; ***ATTO488 fluorophore*** (7 wt%; 10 molecules); biotin content (8 wt%; 27 molecules); tris-NTA ligand (16 wt%; 12 molecules).

(ATTO488: λ_{ex} 500 nm; λ_{em} 520 nm)

Anti-polyHis 800:
~ M_r = 87 kDa; ***Cyanine 7.5 fluorophore*** (2 wt%; 2 molecules); biotin content (8 wt%; 24 molecules); tris-NTA ligand (16 wt%; 11 molecules).

(Cyanine 7.5: λ_{ex} 788 nm; λ_{em} 808 nm)

Storage conditions: Store at 4°C
PROTECT FROM LIGHT!

Application

Anti-polyHis iBodies can be used for a variety of biochemical assays including western blotting (WB).

Following the established procedure, His-tagged proteins immobilized on a membrane can be detected by a quick and simple method which does not require the use of secondary probes nor additional enzyme substrates.

Recommended Western Blotting Procedure

Perform SDS-PAGE and transfer proteins from the gel onto a nitrocellulose membrane (alternatively, a PVDF membrane can be used, however, lower quality results can be expected).

1. Block unoccupied sites on the membrane with 0.55% Casein Blocker (CB) (RT/1hr-12hr).

2. Dilute the iBody in tris-buffered saline solution (TBS) (optimally 1:2,500) and add the incubation buffer (1:100) (RT/20min-12hr).

3. Rinse the membrane with TBS/0.15% Tween 20 (TBST). To wash the membrane, place it into TBST solution and vigorously shake for 5 minutes.

4. Repeat the wash step two more times and finally rinse the membrane with TBS to remove residual detergent.

Choose one of the options for signal detection:

A) Measure the Cyanine 7.5/ATTO488 fluorescence using a suitable instrument.

B) Incubate the membrane with Neutravidin-horseradish peroxidase (Neu-HRP) and capture the chemiluminescence.

Additionally, the anti-polyHis iBodies signal can be amplified by incubating the membrane with neutravidin-fluorophore secondary probe.

Tips and tricks:

a) Optimal working dilution of iBodies is between 1:500 and 1:10,000; The higher the concentration, the higher the signal intensity (and the background).

b) Fluorescence can be measured both prior to and after the Neu-HRP chemiluminescence capture.

c) Drying the membrane after blotting is recommended.

d) Higher incubation times (both during the blocking step and the binding with iBodies) lead to higher signals.

e) Avoid using containers that have been previously used for Coomassie staining.

Contact

In case you are satisfied with the product we would kindly like to ask you to spread the word and cite:

Šácha P, Knedlík T et al. Angew Chem Int Ed Engl. 2016 Jan 8. DOI: 10.1002/anie.201508642

In the opposite case please don't hesitate to contact us on info@ibodies.eu

On behalf of the iBodies team we wish you the best of luck with your experiments!

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