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Application Note for NTA-Tracy 652 (cat.no. 55183)

Nα,Nα-bis-(carboxy-methyl)-L-lysine, Nickel(II) complex, conjugated to Tracy 652-dye (excitation 652nm / emission 677 nm), store at –20°C

Detection of His-p38-MAPK by NTA-Tracy 652 on SDS-PAGE and on Westernblot membrane

Poly-Histidine is one of the most common affinity-tags that can be fused to a protein-of-interest, either N- or C-terminally. The expressed His-tag-fusion-construct is traditionally detected immunologically after gel-electrophoresis and westernblot by anti-His antibody, followed by a secondary antibody that is labelled with horseradish peroxidase or alkaline phosphatase for chemiluminescence detection.
Sigma-Aldrich now offers a Ni-NTA-linked fluorescence dye, which can be directly applied to an SDS-PAGE or a Westernblot membrane for the detection of gel-separated His-tagged proteins by fluorescence imaging.

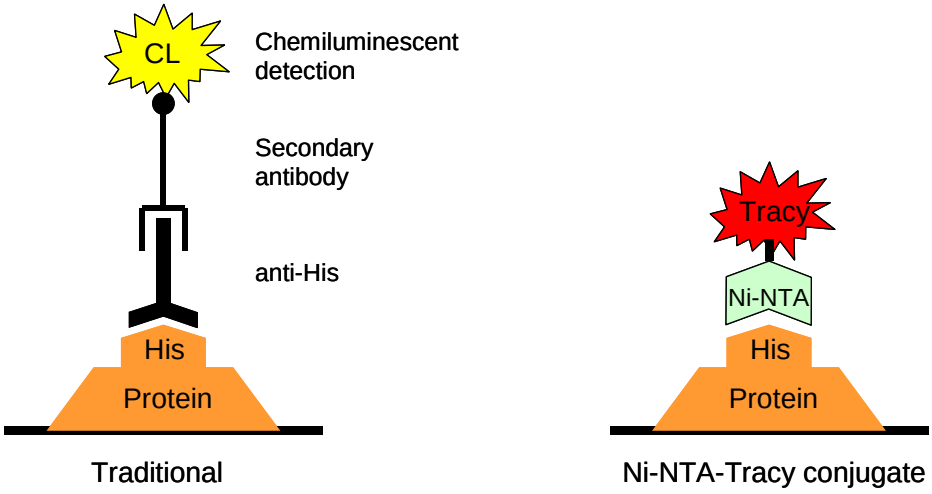

Figure 1. Comparison of protein detection by traditional antibody chemiluminescent immunodetection (left) and Ni-NTA-Tracy detection (right). Traditional immunodetection takes place on a transfer membrane after Western blotting, while the Ni-NTA-Tracy conjugates may be used with either transfer membranes or directly on SDS-PAGE gels after fixing.

In this application note, we use commercially available His-p38-MAPK in different concentrations to demonstrate the use of Ni-NTA-Tracy 652 directly on-gel and on a Westernblot membrane. We recommend to use a low-fluorescence PVDF membrane, e.g. Immobilon-FL.
Ni-NTA-Tracy stock solution is prepared by dissolving 250 µg in 250 µl PBS (pH 7.4), which can then be stored at +4°C in the dark. PBST is prepared by mixing PBS-solution with 0.1% Tween-20.

Detection on SDS-PAGE gel:

- Load the His-tagged protein-of-interest onto an SDS-PAGE
- Run electrophoresis
- Fixation: in 40% Ethanol / 10% acetic acid (1h - overnight)
- Wash: 2x 30min in water
- 1-2h stain: NTA-Tracy 652 (1:1000 – 1:2500 in 25ml PBST)
- Wash: 1-3h in water
- Fluorescence detection (excitation 652nm / emission 677 nm)
  We used a Fuji laser scanner: FLA-3000 (ex 633nm / em 675nm)
- LOD: 50ng/band

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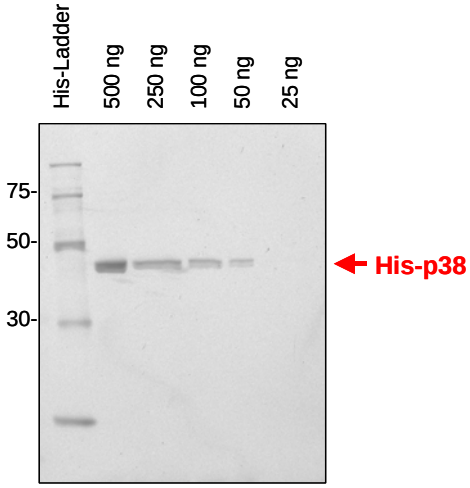

Figure 2. His-tagged p38-MAPK protein (500 ng – 25 ng) was separated on a 4-20% Tris-Glycine SDS-PAGE gel. The gel was fixed overnight in 40% ethanol/10% acetic acid, washed in water and incubated with Ni-NTA-Tracy 652 (1:1000) in the dark. The gel was washed and then imaged using a FLA-3000 Fuji® laser scanner with 633 nm excitation and a 675 nm emission filter. Ni-NTA-Tracy 652 (λ_{ex} 652 nm, λ_{em} 677 nm) is excited in the red region of the spectrum.

Detection on Westernblot membrane:

- Run SDS-PAGE, but no fixation afterwards
- Westernblot transfer onto Immobilon-FL PVDF membrane
- Blocking: 1h - overnight in 5% BSA in PBS
- Short rinse in PBST
- 1-2h stain: NTA-Tracy 652 (1:1000 – 1:2500) in 25ml PBST (dark)
- Wash: 1-3h in PBST (dark)
- Signal-Amplification after drying the membrane
- Fluorescence detection (excitation 652nm / emission 677 nm)
  We used a Fuji laser scanner: FLA-3000 (ex 633nm / em 675nm)
- LOD: 50ng/band

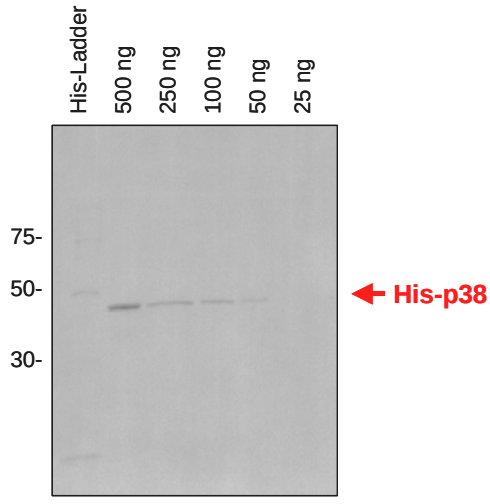

Figure 3. His-tagged p38 protein (500 ng – 25 ng) was separated on a 4-20% SDS-PAGE gel. The protein was transferred to a low-fluorescence PVDF-membrane, blocked overnight with 5% BSA in PBS, rinsed with PBST, and incubated with Ni-NTA-Tracy 652 (1:1000) in the dark. The membrane was washed, dried and then imaged using a FLA-3000 Fuji® laser scanner with 633 nm excitation and a 675 nm emission filter.

A comparison of the procedures for the traditional Western immunoblot and Ni-NTA-Tracy is shown in **Figure 4**. Direct detection on an SDS-PAGE gel is the most convenient as it omits the transfer step. Similar detection limits with Ni-NTA-Tracy conjugates are observed for Western blot membranes compared to SDS-PAGE gels, but without risk of gel damage. In either case, the hands-on time is less than for the traditional antibody technique.

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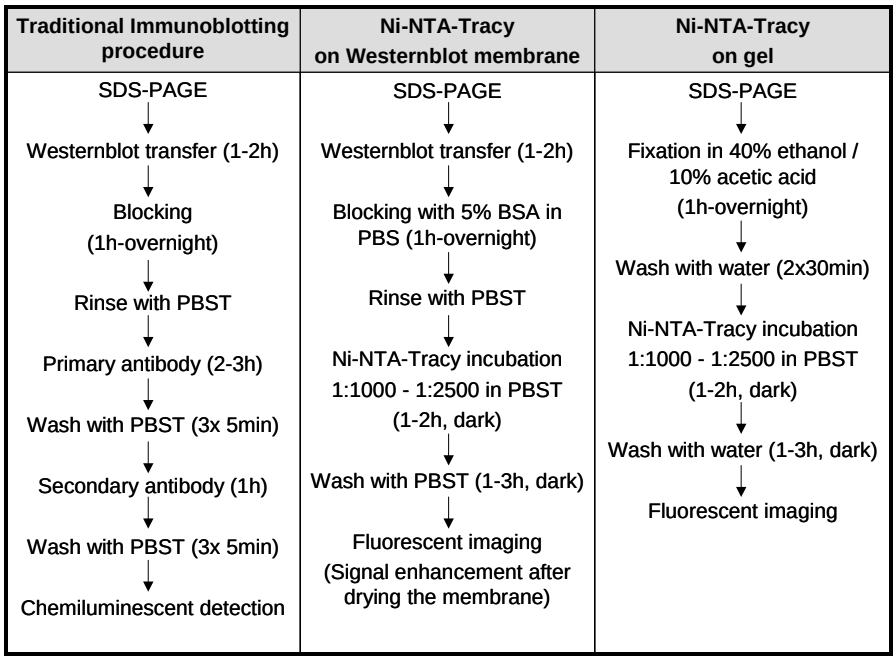

Figure 4. Comparison of the protocols for traditional Western immunoblot, Westernblot using Ni-NTA-Tracy, and direct detection on an SDS-PAGE gel using Ni-NTA-Tracy. Ni-NTA-Tracy stock solution is prepared by dissolving 250 µg in 250 µl PBS.

Ordering Information

Description	Cat. No.	Package size
NTA-Tracy 652	**55183**	250 µg
His-p38-MAPK	**M8057**	50 µg
Laemmli Lysis-Buffer	**38733**	1ml, 5ml, 5x2ml
PBS tablets	**79382**	50 tablets
Tween-20	**P5927**	100ml
Immobilon™-FL PVDF membrane	**05317**	10 ea

Related Products

Description	Cat. No.	Package size
NTA-Atto 647N	**02175**	250 µg
NTA-Atto 550	**94159**	250 µg
NTA-Atto 488	**39625**	250 µg

For additional information on histidine-tagged proteins, visit our homepage at **www.sigma-aldrich.com/hisselect** and **www.sigmaaldrich.com/Area_of_Interest/Biochemicals/Detection.html** for related products.