Quick-Start Protocol April 2016

Detection with Ni-NTA Conjugates

Ni-NTA HRP Conjugate (cat. no. 34530) can be stored lyophilized at 2–8°C for up to 6 months, or for 12 months at –30 to –15°C if not otherwise stated on label. Conjugates can be stored in solution for 1 month at 2–8°C, or for 12 months at –20°C. Recommended storage is in aliquots of stock solution stored at –20°C. Dissolve the lyophilized Conjugate in 500 µl water per vial.

Further information

- *QIA*express *Detection and Assay Handbook*: **www.qiagen.com/HB-2044**
- Safety Data Sheets: **www.qiagen.com/safety**
- Technical assistance: **support.qiagen.com**

Notes before starting

- Prepare western blot (Protocols 1–4), dot blot (Protocol 5) or colony blot (Protocol 6), according to the *QIA*express *Detection and Assay Handbook*.
- See appendix in the *QIA*express *Detection and Assay Handbook* for compositions and preparation of buffers and reagents.
- Ni-NTA Conjugates also form a complex with a 31 kDa molecular-weight standard, bovine carbonic anhydrase, a metalloenzyme with one zinc ion per protein molecule.
- Chemiluminescent substrates are not recommended for use with Ni-NTA Conjugates.
- Best results will be obtained if all steps are carried out on a shaker or rocker platform unless otherwise indicated.

1. Wash western or dot blot membrane twice for 10 min each time with TBS buffer.
2. Incubate for 1 h in 3% BSA in TBS at room temperature.

3. Wash 3 times for 10 min each time with TBS-Tween buffer.
4. Incubate the membrane for 1 h at room temperature in TBS-Tween buffer containing a 1/1000 dilution of Ni-NTA Conjugate stock solution.

   Note: Membranes can be sealed in plastic bags.

   Do not incubate in the presence of BSA, milk or other proteinaceous blocking reagents, chelating reagents or electron donating groups (e.g., $NH4^+$).
5. Wash 3 times for 10 min each time in TBS-Tween buffer at room temperature.
6. Stain with HRP staining solution until the signal is clearly visible (approximately 1–5 min for HRP).

   Do not shake blots during color development.
7. Stop the reaction by rinsing the membrane twice in water.
8. Dry the membrane and photograph as soon as possible.

   Note: The colors will fade with time. The product when using horseradish peroxide substrate is particularly unstable.

Scan QR code for handbook.

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