Quick-Start Protocol April 2016

ELISA with Ni-NTA HisSorb Strips or Plates

Ni-NTA HisSorb Strips (cat. no. 35023) and Ni-NTA HisSorb Plates (cat. no. 35061) should be stored dry at room temperature (15–25°C). They are stable under these conditions for 12 months if not otherwise stated on label.

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

- See appendix in the *QIA*express *Detection and Assay Handbook* for compositions and preparation of buffers and reagents.
- See the *QIA*express *Detection and Assay Handbook* for additional notes on the protocol.
- The interaction of 6xHis-tagged proteins with immobilized nickel ions is pH dependent; binding should be carried out at pH 7.2–7.5.
- The binding capacity is approximately 20 pmol/well for small peptides (20 to 30 amino acids in length) and approximately 10 pmol/well for proteins. An amount of 10 pmol of a 25 kDa protein corresponds to 250 ng.
- Binding can be performed under native or denaturing conditions.
- Binding should be carried out for at least 1 hour at room temperature.
- Best results will be obtained if all steps are carried out on a shaker.
- Suitable negative controls are essential.
- Prepare solutions for HRP reaction immediately before use.
- For higher stringency, the pH of the washing buffer can be lowered to 6.0.

1. Prepare the 6xHis-tagged molecule at various concentrations in PBS/BSA. Alternatively, different dilutions of a cell lysate containing the 6xHis-tagged protein or peptide can be used.

Note: A control without protein should always be included. Concentrations of 6xHis-tagged protein of 0.1–1 µg/ml are recommended. Protein can be immobilized directly from cleared cell lysates.

2. Add 200 µl protein solution to each well, and incubate for 1 h at room temperature.

   Note: Ni-NTA HisSorb Strips and Plates are preblocked and therefore ready for use. The time and temperature necessary for efficient immobilization is dependent on the protein.

3. Wash wells 4 times with PBS-Tween. Soak wells for 10–60 s per wash. Dry the wells by tapping on the strips or plates on paper towels.

4. Add 200 µl of primary monoclonal antibody diluted in PBS/BSA, cover plate and incubate for 1–2 h at room temperature. For higher sensitivity, it may help to perform the antibody binding step overnight at 4°C. Antibody dilution depends on the antibody used. Using a primary antibody conjugated to horseradish peroxidase will decrease the time for the whole assay and lead to even more reproducible results and reduced background. If you are using such a labeled primary antibody, please continue with step 7.

5. Wash wells 4 times with PBS-Tween. Soak wells for 10–60 s per wash. Dry the wells by gently tapping the strips or plates on paper towels.

6. Dilute secondary antibody in PBS/BSA, add 200 µl of the diluted antibody to each well, and incubate at room temperature for 45 min.

   Concentration of secondary antibody should be chosen following manufacturer's recommendations.

7. Wash wells 4 times with PBS-Tween. Soak wells for 10–60 s per wash, and dry the wells by gently tapping the strips or plates on paper towels.

8. Add 200 µl of substrate solution, and monitor color development in a microplate reader.

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