

Immunodetection with Anti-His Antibodies or Anti-His HRP Conjugates (Chemiluminescent Method)

Anti-His HRP Conjugates (cat. nos. 34450, 34460 and 34470) can be stored at 2–8°C for up to 12 months if not otherwise stated on label. Do not freeze. Anti-His Antibodies (cat. nos. 34610 and 34650) can be stored lyophilized for up to 12 months if not otherwise stated on label. In solution, they can be stored for 3 months at 2–8°C, or at –20°C for up to 6 months.

Further information

- *QIAexpress 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) or dot blot (Protocol 5) according to the *QIAexpress Detection and Assay Handbook*.
 - Prepare appropriate reagents according to details shown in Table 1.
 - See appendix in the *QIAexpress Detection and Assay Handbook* for compositions and preparation of buffers and reagents.
 - For compatible chemiluminescent substrates, please refer to manufacturer's recommendations and the *QIAexpress Detection and Assay Handbook*.
1. Wash membrane twice for 10 min each time with TBS buffer at room temperature (15–25°C).
 2. Incubate for 1 h in blocking buffer at room temperature.

3. Wash membrane twice for 10 min each time in TBS-Tween/Triton buffer at room temperature.
4. Wash membrane for 10 min with TBS buffer at room temperature.
5. Incubate in Anti-His Antibody or Anti-His HRP Conjugate solution (1/1000–1/2000 of antibody or conjugate stock solution in blocking buffer) at room temperature for 1 h. Membrane can be sealed in plastic bags.
Note: Do not use buffer containing milk powder for Anti-His Antibody or Anti-His HRP Conjugate dilution.
6. Wash twice for 10 min in TBS-Tween/Triton buffer at room temperature.
7. Wash for 10 min in TBS buffer at room temperature. If using Anti-His HRP Conjugates, proceed to protocol step 10.
8. Incubate with secondary antibody solution for 1 h at room temperature. Either alkaline phosphatase (AP) or horseradish peroxidase (HRP) conjugated anti-mouse IgG may be used.
9. Wash 4 times for 10 min in TBS-Tween/Triton buffer at room temperature.
10. Perform chemiluminescent detection reaction according to the manufacturer's recommendations.

Table 1. Reagents used in chemiluminescent detection of 6xHis tagged proteins

Step	Anti-His Antibody	(Alternative method)	Anti-His HRP Conjugate
Blocking	3% BSA in TBS	1% Casein in TBS	Blocking Reagent in Blocking Reagent Buffer
Anti-His Antibody binding	3% BSA in TBS	1% Casein in TBS	–
Anti-His HRP Conjugate binding	–	–	Blocking Reagent in Blocking Reagent Buffer
Secondary Antibody binding	10% milk powder in TBS	1% Casein in TBS	–



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