Preparation of 6xHis Protein Ladder

The 6xHis Protein Ladder (cat. no. 34705) can be stored lyophilized for 6 months at 2–8°C or in solution for up to 6 months at –20°C. Avoid repeated thawing and freezing.

For more information on protocols and preparation of solutions, please refer to the *QIA*express *Detection and Assay Handbook*, which can be found at www.qiagen.com/handbooks.

For technical assistance, please call toll-free 00800-22-44-6000, or find regional phone numbers at www.qiagen.com/contact.

Notes before starting

- The 6xHis Protein Ladder is used as a molecular weight marker.
- See appendix in the *QIA*express *Detection and Assay Handbook* for compositions and preparation of buffers and reagents.

Table 1. Sizes and amounts of proteins in the 6xHis Protein Ladder

Molecular weight	Amount per lane (ng)*
100	37.5
75	30
50	25
30	25
15	37.5

* When run on a minigel as described below (2.5 µl per lane).

1. Add 500 µl 1x SDS-PAGE sample buffer to the lyophilized 6xHis Protein Ladder.

   Note: It is important to use the buffer recommended. The buffer used must at least contain a suitable reducing agent (e.g., DTT).

2. Allow proteins to dissolve for 30 min at room temperature.

3. Transfer to a microcentrifuge tube and heat for 10 min at 98°C. Store suitable aliquots at –20°C. Always heat the required aliquot immediately before loading the gel.

   Note: It is important to perform this heating step for the duration and at the temperature recommended. Insufficient heating leads to detection of protein aggregates as extra bands.

4. For western blots using mini gels (8 x 8 cm or 10 x 10 cm) and ECL detection, load 2.5–5 µl of the 6xHis Protein Ladder per lane.

   Note: When using larger gels, load correspondingly larger volumes, for example, for gels of 20 x 20 cm use 10–20 µl of 6xHis Protein Ladder.

   To obtain 6xHis Protein Ladder bands of similar signal intensity to protein(s) of interest, load approximately equal amounts of each protein per lane. For example, if detection of 50 ng 6xHis-tagged protein per sample is expected, load 5 µl of dissolved 6xHis Protein Ladder corresponding to approx. 50 ng of each protein (see Table 1).

   If necessary, the dissolved 6xHis Protein Ladder can be diluted with 1x SDS-PAGE sample buffer just before loading the gel.

For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit handbook or user manual.

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