proflo His-tag quick test

Competitive immuno-chromatographic test for detection of histidine-tagged recombinant proteins in cell lysates or purified protein preparations.

Cat. No.: **PRLFHIS**
Content: **25 Lateral Flow Tests**
Storage: **2 - 8 °C**
Version: **01**

For research use only!

1. Introduction

For purifying recombinant proteins, the histidine tag (His-tag) is the most widely used peptide tag with usually 6 – 10 consecutive histidines at the N- or C-terminus of a target protein. Purification of His-tagged proteins is done by using immobilized metal ions like nickel or cobalt in a variety of different buffer conditions.

Proflo His-tag quick test uses a competitive assay for checking and monitoring the presence of His-tagged recombinant proteins in small-scale screening experi-ments, during scale-up in larger scale as well as in fractions during the purification process. The quick and reliable identification of samples with the highest content of His-tagged proteins will save time for following downstream steps, avoiding other time-consuming analytic techniques like SDS-PAGE and/or Western Blot.

The test is a semi-quantitative assay with a concentration range of **2 – 50 µg/ml** (for a 25 kDa protein). The use of a positive control in the same buffer matrix with known concentration and similar size of your recombinant protein will allow an estimation of the concentration of the unknown sample. The comparison of different samples with unknown concentrations will lead to identification of samples with higher concentrations.

2. Test Principle

The test is based on a competitive reaction between a specific anti-His-tag antibody bound to gold particles (capture antibody complex) localized at the bottom of the test strip and a His-tagged protein immobilized on the test line of the test strip (Figure 1).

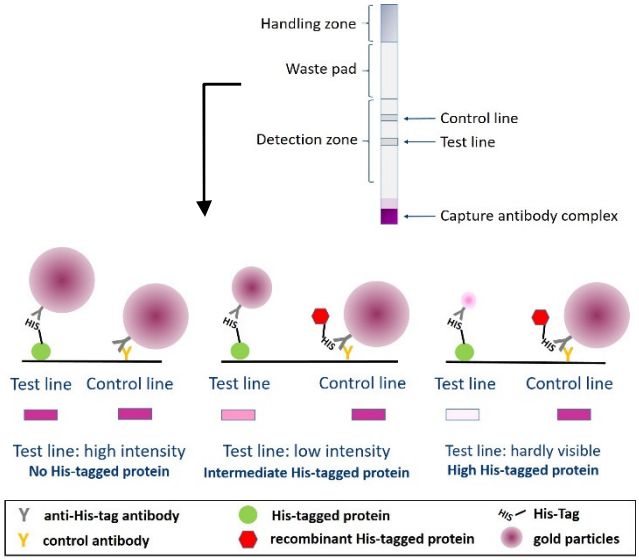

Figure 1

After applying a sample to the test strip, the capture antibody complex binds the His-tagged recombinant protein present in the sample and flows by capillary force across the strip membrane. Capture antibody complexes that are not bound to recombinant proteins are captured by the His-tagged proteins at the test line.

Capture antibody complexes that are not captured by the His-tagged proteins at the test line bind to specific control antibodies located at the control line. **Thus, with increasing amount of His-tagged protein in the sample, the test line staining will appear weaker.** If the test line is not visible due to saturation of binding sites on the capture antibody complexes, a new test strip should be used with a higher dilution of the sample if analysis or comparison of samples with higher concentrations is intended. **Depending on the size of your protein and accessibility of the His-tag, the intensity of the test line can vary significantly.**

Note:
The control line is not dependent on the amount of His-tagged protein in your sample and should always appear as a strong purple line. Without a clearly visible control line, the test is not valid. The binding to the control antibody could be inhibited in such cases. The test should be repeated with a higher dilution of the sample.

3. Required Material

- Precision pipets
- Pipet tips
- Optional: Phosphate buffered saline (PBS)

4. Test Kit Content

Test strips	25 His-tag lateral flow test strips in plastic container
Buffer	Dilution buffer, 2 x 2 ml
Reaction tubes	25 x 2 ml tubes

5. Preparation of Reagents

Prior Prior to use, allow the test strips to reach room temperature (RT, 20 – 26°C). **Immediately after taking out the required number of strips, close the plastic container with the lid containing the desiccant to avoid exposure of the strips to moisture and light. Please use gloves and touch the strips only at the handling zone (Figure 1).**

For testing crude lysates or purified samples please note, that the test should be performed shortly after sample preparation in order to ensure the correct analysis of His-tagged protein in the lysate. Degradation by proteases could result in lower levels of His-tagged protein.

Sample preparation (Figure 2, next page)

1. Pipet **95 µl dilution buffer** into the 2 ml reaction tubes included in the kit.
2. Add **5 µl** of your cell lysate or purified fraction (His-tagged recombinant protein) to the 95 µl and mix thoroughly with the pipet.

   If your sample contains higher concentrations of His-tagged protein (e.g. according to the absorption curve during a purification run), you can pre-dilute your sample in PBS accordingly.
3. Place a single test strip into each reaction tube with the diluted sample and let it stand for 10 min until a clearly visible, sharp control line appears.

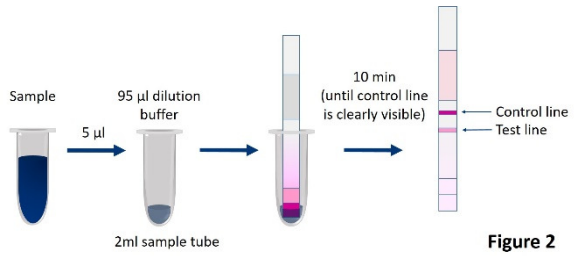

Figure 2

6. Storage & Stability

Store the test kit and components at 2 - 8°C.

Stability after opening:

Test strips: 4 weeks at 2 - 8°C

Dilution buffer: 4 weeks at 2 - 8°C

7. Documentation of Results

The optical analysis of the test strips can be done within 30 minutes after incubation of the test strips and before the test strip is completely dry. A high intensity of the test line indicates very low concentration or absence of His-tagged recombinant proteins in your samples, a low intensity indicates an intermediate His-tagged protein concentration, and a hardly visible test line indicates a high His-tagged protein concentration (Figure 3, see page 3). In case of different samples with unknown concentrations, simple comparison of the test lines by eye will provide sufficient accuracy for identifying the concentration differences between your unknown samples.

Depending on the size of your His-tagged recombinant protein and the accessibility of the His-tag, the intensity of the test line can vary significantly.

If available, a reader for lateral flow tests can be used to scan the strips for a more detailed analysis.

Alternatively, for documentation and analysis purposes, a flat-bed scanner can be used to scan multiple strips in parallel taped on a sheet of paper. Further analysis can be performed using an image analysis software to get semi-quantitative results for your samples.

8. Test Validity

The upper control line must be clearly visible in all cases (positive control, negative control, unknown samples) in order to confirm the migration of the His-tag antibody-gold particle complexes over the whole detection area of the strip.
If the staining of the control line is very weak or absent, the test is not valid.

9. Test Characteristics

The detection range of the strips is 2 – 50 µg/ml (for a 25 kDa protein). With increasing molecular weight of your protein, more protein may be needed to achieve the same intensity as for smaller proteins.

10. General Information

Transport damages
If a kit is considerably damaged, please contact the manufacturer or local distributor. Do not use damaged components for test procedure. Such components or kits should be stored at 2 – 8°C until the complaint is handled.

Precautions
The buffer contains preservatives. Do not swallow! Avoid any contact with skin or mucous membranes.

Safety data sheet available on request.

Disposal
Products and packaging must be disposed of in compliance with the respective national regulations.

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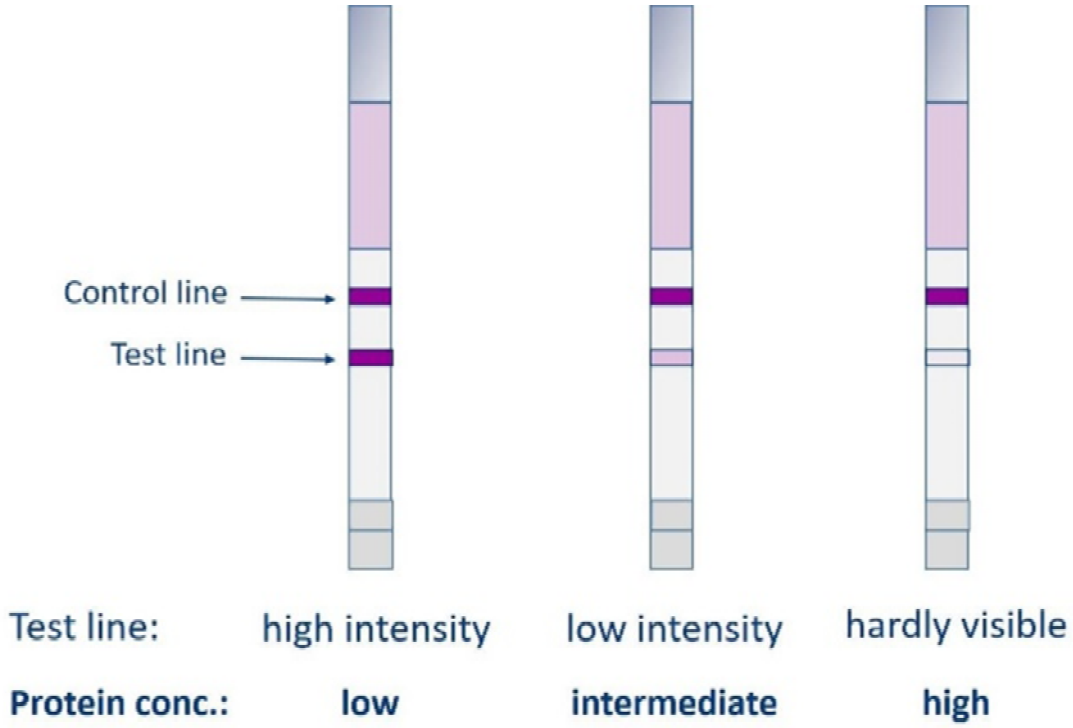
Figure 3


Control line
Test line
Test line:
high intensity
low intensity
hardly visible
Protein conc.:
low
intermediate
high