QoolAbs, Inc.
4186 Sorrento Valley Blvd, Suite D/E, San Diego, CA 92121
Tel: (858) 348-0988
Website: www.qoolabs.com email: support@qoolabs.com

INSTRUCTIONS

37ExpressVue® His Tag Rapid Detection

(Patent Pending)

Catalog number for His Tag Rapid Tests (2-Line): 115100-020, 115100-050, 115100-096, 115100-005

Catalog number for His Tag Rapid Tests (4-Line): 115102-020, 115102-050, 115102-096, 115102-005

Related Materials (Sold Separately)

37ExpressVue® HIS Tag Positive Controls: Ready to use, 100ug/ml, 0.5ml, Catalog Number 915101-001

Peptide epitope tags are widely used in recombinant protein expression for detection and purification. Poly Histidine (His) tag is one of most commonly used epitope tags due to its smaller size and easy purification of the tagged protein with metal affinity chromatography. Detection of protein using gel electrophoresis/Western Blotting or ELISA methods can be cumbersome and time consuming and requires skilled handling. The 37ExpressVue® His Tag Rapid Tests detect recombinant protein directly from cell culture media or lysate without any special instruments or sample handling.

Principles of the Tests

The 37ExpressVue® His Tag Rapid Tests are **COMPETITION** immunochromatographic membrane assays that use antibodies to detect epitope tagged proteins in cell culture and lysate. For the 2-line tests, a His tagged protein antigen and a secondary antibody are immobilized on a membrane support as two distinct lines: a test line (T line) and a control line (C line). A His tag specific antibody is labeled with colored particles (colloidal gold nanoparticles) to allow visualization of the formation of immunocomplex composed of the antibodies and the epitope tag (Figure 1, top).

In a valid test, the labeled antibody will be captured by the secondary antibody on the control line and become visible in all tests. If the sample has NO His tagged protein, the labeled antibody will be captured by the printed antigen on the test line and form a visible red line – both test and control lines are visible – indicating a negative sample (Figure 1, Middle). If the sample has His tagged protein, it will compete the antibody binding with the antigen on the test line, therefore the test line will be **INVISIBLE** or appear as a line with reduced density (Figure 1, Bottom). The density of the test line **INVERSELY** correlate with the amount of His tagged protein present in the sample.

Figure 1. Diagrams illustrating competition LFIA. Top: Layout of an unused test strip. Middle: When a sample without His tag is applied, two red lines will appear. Bottom: When a sample with His tag is applied, one control line will appear.

- Gold labeled Anti-His Antibody
- Test Line immobilized His tagged protein
- Control Line immobilized 2nd Antibody
- His Tagged protein in sample

In the 37ExpressVue® His Tag 4-Line Rapid Test, His tagged protein antigen of 3 different concentrations were immobilized on the membrane as 3 test lines, in addition to the control line. Therefore, in the negative samples without His-tagged protein, four distinct lines will appear on the test strip after testing. Increased concentration of His tagged protein in samples will cause the disappearance of test lines from low concentration of immobilized antigen to high concentration. The His Tag 4-Line rapid tests may be used in semi-quantitative determination of His tagged protein.

Intended Use

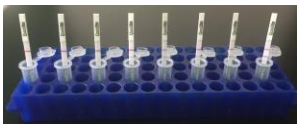
The 37ExpressVue® Test strips may be used in monitoring recombinant protein expression in a variety of applications, such as optimization of protein expression conditions, real time monitoring of protein expression, determining dose response of inducer in protein expression, monitoring change of protein expression levels in response to environments, such as

temperature, nutrient or oxygen level, etc. They may also be used in high yield clone selection, quality control for protein purification steps.

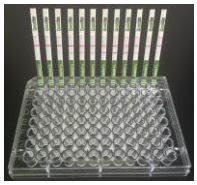
Test Procedure

1. All tests are performed at room temperature. Allow the package of test strips warm to room temperature for 15 minutes prior to taking the strips out of the moisture barrier bags to avoid condensation.
2. Pipette ~200 ul of sample into an Eppendorf tube or ~100 ul into a well of 96-well microtiter plate. Hold the "QoolAbs" logo end of the strip, dip the other end of the strip (with stripped lines) into the sample, making sure only the white pad below the green colored lines is immersed into the sample (Figure 2A and 2B).
3. 15 - 20 minutes later, read the test result (Figure 2C and 2D). Results read before or after this time frame may be inaccurate.

⚠ ***NOTE:*** **It is recommended to test a negative control to get a background reading of the test and control lines each time using the His-tag rapid test. Each package of this product is provided with an extra set of test strips for this purpose.**

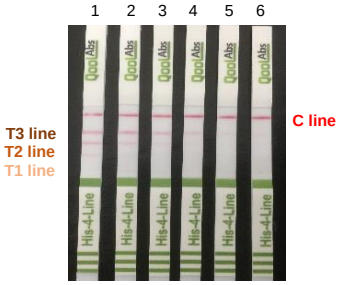


2A



2B

2C



2D

Result Interpretation

His Tag Rapid Tests (2-Line, Cat # 115100): At the end of the reaction (about 15 minutes), both the control line and the test line will be clearly visible in the negative control sample (strip #1 in Figure 2C). In test samples, if both the control line and the test line appear at similar density to those of the negative control, it indicates that there might be no detectable His tagged protein. His tagged protein at higher concentration will cause reduced density or disappearance of the test line (Figure 2C, strips 2-6, samples containing N-terminal 6xHis tagged protein at 50ng/ml, 200ng/ml, 600ng/ml, **1000ng/ml**, 4000ng/ml in PBST containing 0.1% BSA). The density of the test line **INVERSELY** correlate with the amount of His tagged protein present in the sample. When a negative control is tested, the test line may appear slower than the control line and at a lower density in the first few minutes of the reaction.

His Tag 4-Line Rapid Tests (Cat # 115102): At the end of the reaction (about 15-20 minutes), the control line and all three test lines will be clearly visible in the negative control sample (strip #1 in Figure 2D). In test samples, increased concentration of His tagged protein will cause disappearance of the test lines in the sequence from T1 to T3 (Figure 2D, strips 2-6, samples containing N-terminal 6xHis tagged protein at 200ng/ml, **400ng/ml**, **1000ng/ml**, 2000ng/ml, **5000ng/ml** in PBST with 0.1% BSA). The density of the test lines **INVERSELY** correlate with the amount of His tagged protein present in the sample.

The test strips have been tested with fusion proteins containing 6xHis at either C-terminus or N-terminus, and 8xHis tag at the C-terminus. Test sensitivity may vary with each fusion protein depending on the location and length of the His tag due to variations in binding affinity of the anti-His tag antibody to each fusion protein. The rapid tests work in a wide range of buffer systems. However, it is recommended to test the sample buffer and positive/negative controls.

Precautions

1. Keep test strips sealed in its foil pouch until just before use.
2. Do not re-use the test strips.
3. Do not use the strips past its expiration date.
4. Excessive air circulation (i.e. air conditioners, fans, etc.) can slow the flow of the sample. During testing, protecting the devices from excessive air flow is recommended.

Storage and Stability

Store test strips dry at 4°C. Do not freeze. After opening, the unused strips should be stored in a desiccator and use within one week; Or, for test strips packed in re-closable aluminum bags, unused strips should be kept in the sealed bag with the supplied desiccants and use within one week.