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INSTRUCTIONS

37ExpressVue® DYKDDDDK Tag Rapid Detection

(Patent Pending)

Catalog number: 115200-020, 115200-050, 115200-096, 115200-005

Related Materials (Sold Separately)

37ExpressVue® DYKDDDDK Tag Positive Control: 100ug/ml, 0.5ml, Catalog Number 915201-001

DYKDDDDK Tag (Trademark of Sigma as FLAG) is one of most commonly used epitope tags in recombinant protein expression for detection and purification due to its smaller size and easy purification of the tagged protein with anti-DYKDDDDK 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® DYKDDDDK Tag Rapid Test detects recombinant protein directly from cell culture media or lysate without any special instruments or sample handling.

Principles of the Procedure

The 37ExpressVue® DYKDDDDK Tag Rapid Tests are **COMPETITION** immunochromatographic membrane assays that use antibodies to detect epitope tagged proteins. For a standard 2-line competition test, a 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 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 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 tagged protein, it will compete the antibody binding with the tagged 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 tagged protein present in the sample.

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

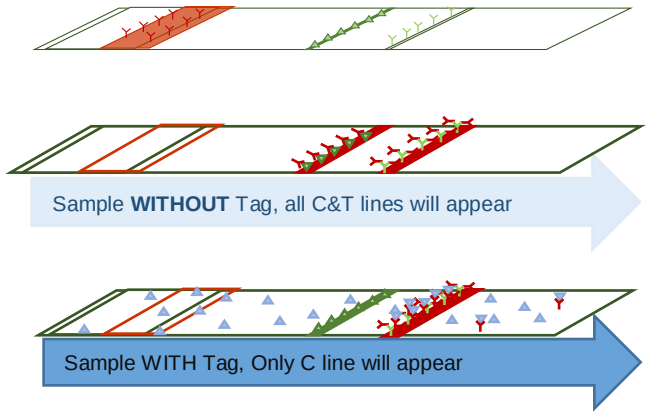

Figure 1. Diagrams illustrating competition LFIA. Top: Layout of an unused test strip. Middle: When a sample without DYKDDDDK tag is applied, four red lines will appear. Bottom: When a sample with enough tagged protein is applied, one control line will appear.

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

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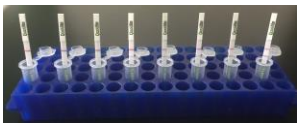
Intended Use

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.

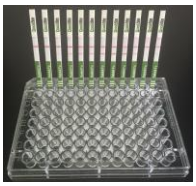
Test Procedure

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). 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 rapid test. Each package of this product is provided with an extra set of test strips for this purpose.**



2A



2B

2C

Result Interpretation

When a negative control is tested, the control line and all three test lines will be clearly visible in the negative control sample (strip #1 in Figure 2C) at the end of the reaction (about 15-20 minutes). The test lines may appear slower than the control line and at a lower density in the first few minutes of the reaction. In test samples, increased concentration of DYKDDDDK tagged protein will cause disappearance of the test lines in the sequence from T1 to T3 (Figure 2C, strips 2-5, samples containing C-terminal FLAG Tag protein at **4ug/ml**, 8ug/ml, **12ug/ml**, **60ug/ml**, in PBST with 0.1% BSA). The density of the test lines **INVERSELY** correlate with the amount of Tag protein present in the sample. Test sensitivity may vary with each tagged protein due to the variation in protein structure and binding affinity of the anti-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.