
INSTRUCTIONS

37ExpressVue® Rapid Tests for DYKDDDDK-HIS Double Tag (Patent Pending)



Cat# 115201-096, 115201-050, 115201-020

Related Materials (Sold Separately)

37ExpressVue® DYKDDDDK-HIS Positive Controls, ready to use, 0.5ml. Cat # 915202-001

The tandem affinity purification (TAP) technique employs two sequential affinity purification steps and thus reduces the chance of contaminants and broken fragments retaining in the eluate. DYKDDDDK (FLAG)-HIS double tag is popular for this purpose in recombinant protein expression and production. Detection of protein, which is necessary during these processes, using gel electrophoresis/Western Blotting or ELISA methods can be cumbersome and time consuming and requires skilled handling. The 37ExpressVue® DYKDDDDK-HIS Test detects double tagged protein with DYKDDDDK and HIS at each end directly from cell culture media or lysate without any special instruments or sample handling. The test is completed in 5 to 10 minutes.

Principles of the Procedure

37ExpressVue® DYKDDDDK-HIS Test is an immunochromatographic membrane assay that uses antibodies to detect DYKDDDDK-HIS double tagged proteins. A detection antibody and a control antibody are immobilized on a membrane support as two distinct lines. A capture antibody is labeled with colored particles (colloidal gold nanoparticles) to allow visualization of the formation of immunocomplex between the antibodies and the DYKDDDDK-HIS double tagged protein.

To perform the test, the strip is dipped into samples of cell culture media, cell lysate or other fractions. DYKDDDDK-HIS double tagged protein present in the sample binds the gold-labeled capture antibody, which is specific to DYKDDDDK. The antigen-antibody-gold complexes migrate along the test strip, where they are captured by the immobilized detection antibody for HIS tag, forming the Test Line (T). Immobilized control antibody captures the overflow complexes, forming the Control Line (C). The appearance of both a T Line and a C Line indicates the presence of tagged protein in the sample.

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 and/or oxygen level, etc. The double tag rapid tests are also useful in analyzing sample fractions after enzymatic cleavage of tagged proteins.

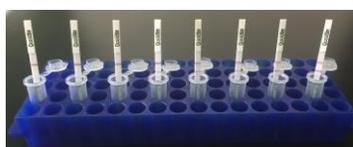
Test Procedure

1. All tests are performed at room temperature. Allow the package of strips warm to room temperature for 15 minutes prior to taking test strips out of the moisture barrier bags to avoid condensation.

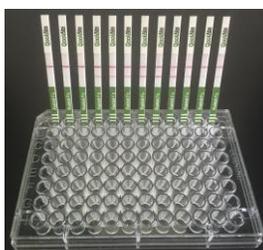
- Pipette ~200 ul of sample into an Eppendorf tube or ~120 ul into a well of 96-well microtiter plate. Hold the “QoolAbs” logo end of the strip, dip the other end of the strip (with stripped lines) into the sample, making sure only the white pad below the green colored lines is immersed into the sample (Figure A and B).

NOTE: The linear detection range of the DYKDDDDK-HIS test strips is 6.25 ng/ml to 50 ng/ml. The test line is most visible for samples with the concentration between 6.2 ng/ml and 10 ug/ml. To test samples with high concentrations of DYKDDDDK-HIS tagged protein, dilute the samples with PBS prior to testing to avoid hook effect (loss of signal at high concentrations of target protein).

- 5-10 minutes later, read the test result (Figure C). Results read before or after this time frame may be inaccurate.



A



B



C

Sample Tests and Result Interpretation

Purified protein with DYKDDDDK (N-terminal) and HIS tag (C-terminal) was diluted to various concentrations with sample buffer (PBST containing 0.1% BSA). Each diluted sample was tested with a DYKDDDDK-HIS rapid test strip. As shown in Figure C, samples for strips 1 to 14 contained DYKDDDDK-HIS tagged protein at the following concentrations: 0, 6.25ng/ml, 12.5ng/ml, 25ng/ml, 50ng/ml, 100ng/ml, 200ng/ml, 400ng/ml, 1.0ug/ml, 10ug/ml, and 100ug/ml. There was no background signal when no DYKDDDDK-HIS protein was present in the sample (strip 1). The low detection limit of the DYKDDDDK-HIS test strips is 6.25 ng/ml of DYKDDDDK-HIS tagged protein (strip 2). The linear detection range of the test strips is 6.25 ng/ml to 50 ng/ml (strips 2 to 10). The test line is most visible for samples with the concentration between 6.2 ng/ml and 10 ug/ml (strips 2 to 10). When protein concentration is expected to be higher than the upper limit of the linear range, it is recommended to try 2-3 different dilutions of the original samples to avoid hook effect (loss of signal when there is too much test targets present in the sample, strips 10 and 11).

Precautions

- Keep test strips sealed in its foil pouch until just before use.
- Do not re-use the test strips.
- Do not use the strips past its expiration date.

Storage and Stability

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