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INSTRUCTIONS

37ExpressVue™ Rapid Tests for MBP Tag

(Patent Pending)

Cat# 112200-096, 112200-050, 112200-020

Related Materials (Sold Separately)

37ExpressVue™ MBP Positive Controls, ready to use, 1ml, Cat # 912201-001

Maltose binding protein (MBP) is a popular tag for recombinant protein expression and production. Detection of protein using gel electrophoresis/Western Blotting or ELISA methods can be cumbersome and time consuming and requires skilled handling. The 37ExpressVue™ MBP Test detects MBP tagged protein 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™ MBP Test is an immunochromatographic membrane assay that uses antibodies to detect MBP tagged proteins in cell culture and lysate. 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 MBP tagged protein.

To perform the test, the strip is dipped into samples of cell culture media or lysate. MBP tagged protein present in the sample binds the gold-labeled capture antibody, which is specific to MBP. The antigen-antibody-gold complexes migrate along the test strip, where they are captured by the immobilized detection antibody, 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.

Test Procedure

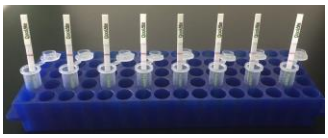
1. All tests are performed at room temperature. Allow the package of strips warm to room temperature for 15 minutes prior to taking test strips out of the moisture barrier bags to avoid condensation.
2. Pipette ~200 ul of sample into an Eppendorf tube or ~120 ul into a well of 96-well microtiter plate. 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 : To test samples with high concentrations of tagged protein, dilute the samples with PBS prior to testing. The lower detection limit of the MBP test strips is 1 ng/ml of MBP. The linear detection range of the MBP test strips is 10 ng/ml to 400 ng/ml. The test line is most visible for samples with concentration

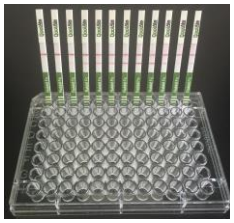
between 10 ng/ml and 10 ug/ml. When protein concentration is expected to be higher than the upper limit of the linear range, it is recommended to try 2-3 different dilutions of the original samples to avoid hook effect (loss of signal when there is too much test target present in the sample).

⚠ MBP is endogenously expressed in many *E.coli* hosts such as BL21. At 1000, 100, and 10ug/ml total protein concentration, endogenous MBP in BL21 lysate is clearly detectable and will interfere with the detection of MBP fusion protein (figure C, strips 1, 2, 3). Therefore, when detecting MBP directly from *E.coli* lysates of these host strains, it is recommended to dilute the sample so that the total protein concentration is ~1ug/ml or lower to avoid the background caused by endogenous MBP (figure C, strip 4). MBP gene was deleted in host strain ER2507, in which there are no detectable background MBP expression at 1000, 100, 10 or 1ug/ml total protein concentration (figure C strips 5, 6, 7 and 8).

3. 5-10 minutes later, read the test result. Results read before or after this time frame may be inaccurate.



A



B

C

D

Sample Test Results

BL21 cells containing a MBP expression vector were induced for various period of time at 30ºC. 1ml fractions of the culture were collected at each time point. Cells were harvested by centrifugation and resuspended with PBS and sonicated to get the whole cell lysates. Protein concentration of each lysate was determined by Bradford assay. All samples were diluted to 1ug/ml of total protein concentration. MBP was detected with the MBP Rapid Detection Test Strips (figure D). There were no detectable MPB at 0, 5 and 10 minutes (strips 1, 2 and 3); MBP was detectable as early as 20 minutes after induction (strip 4), and increased as the culture continued (strips 5 to 14, time points of 30, 40, 60, 90, 120, 150, 180, 210, 240 minutes and overnight).Strip 15 was the result of a lysate from a MBP deleted host strain ER2507. The sample for strip 16 was from a lysate of BL21 without expression vector at 1ug/ml total protein concentration.

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.

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.