ProXpress (Flag-Tag)-Competitive Rapid Test Card Manual

Cat. No.: HX002323-5

Unit size: 5

Expected Use

Rapidly detects Flag-tagged protein products obtained from prokaryotic and eukaryotic expression systems.

Detection Principal

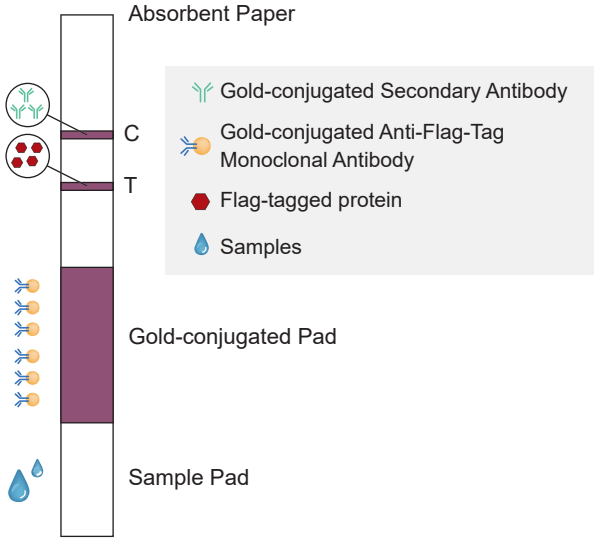
This product is a semi-quantitative protein detection reagent that relies on the use of a colloidal gold-based lateral flow assay. The foundation of this product is comprised of various components, such as a sample pad, a gold-conjugated pad, a chromatography membrane, and absorbent paper.

When administering the assay, users will add a drop of the test sample onto the sample pad, which then undergoes chromatography via capillary effects. In cases where no Flag-tagged protein is present in the sample, the gold-conjugated anti-Flag-Tag antibody will move with the laminar flow to the T line (also referred to as the test line). From this point, the antibody will then bind specifically to the immobilized Flag-tagged protein on the T line, forming a purplish-red band.

However, when Flag-tagged protein is present in the test sample, the gold-conjugated anti-Flag-Tag antibody will bind solely to the Flag-tagged protein, leading to an increase in antibody binding as more Flag-tagged protein is introduced. As the laminar flow moves towards the T line, there will be less free gold-conjugated anti-Flag-Tag antibody present to bind to the immobilized Flag-tagged protein, which in turn results in a possible faint purple-red band or no band.

Moving onto the control line (C line), the gold-conjugated anti-Flag-Tag antibody will be captured by the immobilized gold-conjugated secondary antibody on the C line, resulting in a purplish-red band. To determine the presence or absence of Flag-tagged protein in the sample being tested, users need to read whether both the T line and C line are colored and the intensity of the coloration.

In summary, this reagent relies on a gold-conjugated lateral flow assay that can detect Flag-tagged protein based on chromatography via capillary effects. By carefully observing the coloration of both the T and C lines, users can determine the presence or absence of the protein they are testing for.



Schematic Diagram of the Test Principle

Package Contents

1. Flag-Tag (competitive) rapid test cards
2. ProXpress dilution buffer
3. Instructions

Storage & Validity

Stored in a cool place, 4~30°C, do not freeze, avoid direct sunlight. Valid for 12 months.

Instructions

1. Begin by letting the test card equilibrate to room temperature before conducting any further steps. This is crucial to ensure accurate results.
2. The proper pre-dilution of test samples is essential to obtain reliable results. To maintain the concentration of the test protein between 1-10 μg/ml, make sure not to exceed or fall below this concentration range. Concentrations higher than 10 μg/ml can result in weak or barely visible color development of the T line, leading to distorted protein concentration interpretation. Conversely, low protein concentrations below 1 μg/ml can weaken the color development of the T line compared to the negative control T line, resulting in false-negative results.
If you know the concentration of the target labeled protein, you can directly dilute the sample with ProXpress dilution buffer to a concentration of 3 μg/ml. If you don't know the concentration, you can perform a 25-fold dilution of the sample from a bacterial lysate or a 5-fold dilution for a sample from mammalian cell supernatant or lysate with ProXpress dilution buffer. Ensure thorough mixing by vortexing.
3. Draw 20 μl of the pre-diluted test sample using a micropipette and dispense it into the sample well.
4. Next, add 50 μl of ProXpress dilution buffer to the sample well. You can accomplish this by gently adding two drops vertically from the dropper.
5. After completing the previous steps, read the results after 10-15 minutes.

Note: If the test result of the sample after pre-dilution shows very weak color development and is difficult to discern with the naked eye, this may indicate the possibility of an excessive sample concentration. In such cases, it is best to repeat the test with a second 10-fold dilution of the pre-diluted sample. If visible bands appear on the T line, it confirms that the initial weak color development was indeed due to excessive protein concentration. If there is suspicion that the color difference between the T line and the negative control T line is challenging to distinguish due to low protein concentration, the dilution factor in the pre-dilution step can be reduced to 3-fold. However, we do not recommend a dilution factor lower than 3-fold, as testing results in such cases can be influenced by complex components in the protein solution and lead to reduced testing accuracy.

Overall, ensure to follow the steps above to obtain reliable and accurate results.

Product Performance Indicators

The test strip has a minimum detection limit of 1 μg/mL. As the concentration of the Flag-tagged protein in the test sample reaches 1 μg/mL and higher, the intensity of the T line's color weakens, resulting in a faint purplish-red band or even no coloration.

In cases where the Flag-tagged protein concentration falls within the range of 1 μg/mL to 10 μg/mL, the color depth of the T line displays a negative correlation with the protein concentration. As the protein concentration increases within this range, the T line's color appears lighter.

When the Flag-tagged protein concentration in the test sample surpasses 30 μg/mL, the color of the T line completely fades out.

Results Interpretation

1. **Negative Results:** Color development of both the C and T lines. The intensity of the T line color matches that of the negative control.
2. **Positive Results:** The C line is colored, but the T line is not colored or is significantly weaker than the negative control.

Note: When no Flag-tagged protein is present, the T line exhibits the deepest color. As the Flag-tagged protein content increases, the color of the T line gradually diminishes until it disappears.

3. **Invalid Result:** No color is observable on the C line, regardless of the presence of color on the T line. This indicates an ineffective reagent, rendering the test invalid.

ProXpress (Flag-Tag)-Competitive

Test card color rendering display diagram

Additional Information

Substance	Compatible Concentration	Substance	Compatible Concentration
NaCl	0.25M	EDTA	5mM
Urea	0.4M	Glycerol	10%
TritonX-100	1%	KCl	0.25M
Tween-20	1%	CHAPS	1.0%
SDS	0.20%	RIPA	100%
NP-40	1%	—	—

Troubleshooting

Observation	Possible Cause	Recommended Action
No visible weakening of the test line compared to the negative control	The sample did not contain Flag-Tag protein	Verify that the correct test card is used.
		Verify presence of Flag-Tag protein via alternative method (e.g. ELISA or Western Blot).
Low intensity test lines	Sample concentration above the upper limit of the working concentration range	Repeat the test with a second dilution of the test sample.
No control line detected	Incorrect operation	Repeat the procedure according to the instructions and make sure that the concentration of lysis and extraction reagents added to the sample is within the recommended range.
	Test card exceeds expiration date	Use the test card within the expiration date.

21 August 2023