

# ProXpress (Flag-His-Tag) Rapid Test Card Manual

Cat. No.: HX002321-5

Unit size: 5

## Expected Use

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

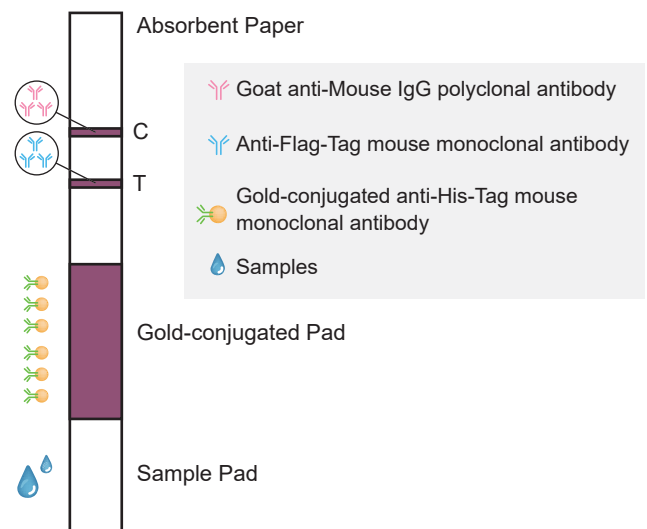
## Detection Principle

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. When Flag-His-tagged protein is present in the test sample, the gold-conjugated anti-His-Tag mouse monoclonal antibody will bind solely to it.

As the complex laminar flow moves towards the T line, it is captured by the immobilized anti-Flag-Tag mouse monoclonal antibody, forming a purplish-red band. Moving onto the control line (C line), it is captured by the immobilized Goat anti-Mouse IgG polyclonal antibody, forming a purplish-red band.

In summary, this reagent relies on a gold-conjugated lateral flow assay that can detect Flag-His-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-His-Tag 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 4-500 ng/ml, make sure not to exceed or fall below this concentration range. Concentrations higher than 500ng/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 4 ng/ml can weaken the color development of the T line compared to the negative control T line, resulting in difficult to read with naked eye.  
If you know the concentration of the target labeled protein, you can directly dilute the sample with ProXpress dilution buffer to a concentration of 100 ng/ml. If you don't know the concentration, you can perform a 50-fold dilution of the sample from a bacterial, mammalian, yeast and insect cell lysate with ProXpress dilution buffer. Ensure thorough mixing by vortexing.
3. Draw 20  $\mu$ l of the pre-diluted test sample using a micropipette and dispense it into the sample well.
4. Next, add 50  $\mu$ l 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, or the concentration is seriously lower than expected, then there is a possibility that the concentration of the sample is too high (e.g., the concentration of the sample is more than 1 mg/ml), it is recommended that the pre-diluted sample should be diluted by a second time by 100-fold and repeat the test. If there is a visible band in the T line, it means that the protein concentration is too high; if no visible band is observed, it means that your test material does not contain Flag-His-tagged protein or its content is lower than 200 ng/ml; if you suspect that the protein concentration is too low to cause the weak color development, you can reduce the dilution factor to 10 times. We don't recommend pre-dilution of less than 10 times, because in this case, the results will be easily interfered by the complex components in the protein solution, resulting in a decrease in the accuracy of detection.

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 4 ng/mL.

In cases where the Flag-His-tagged protein concentration is within the range of 4 ng/mL to 500 ng/mL, the color depth of the T line displays a positive correlation with the protein concentration. As the protein concentration increases within this range, the T line's color appears deeper.

When the Flag-tagged protein concentration in the test sample surpasses 1000 ng/ml, the T line is maintained at a high intensity and there is no significant reduction in the coloration due to excessive amounts of the test substance.

## Results Interpretation

1. **Negative results:** Color development of the C line, with no observable color on the T line.

2. **Positive results:** Color development of both the C and T lines.

Note: when the sample contains Flag-His-tagged protein, as the Flag-His-tagged protein content increases, the color of the T line gradually enhances.

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-His-Tag)



Test card color rendering display diagram

## Additional Information

Substance	Compatible Concentration	Substance	Compatible Concentration
NaCl	1.5M	EDTA	7.5mM
Urea	0.4M	Glycerol	10%
TritonX-100	1%	KCl	1.5M
Tween-20	1%	CHAPS	1.0%
SDS	0.20%	RIPA	100%
NP-40	1%	—	—

## Troubleshooting

Observation	Possible Cause	Recommended Action
No test lines detected	Sample did not contain Flag-His-Tag protein	Verify correct test card is used.
		Verify presence of Flag-Tag protein via alternative method (e.g. ELISA or Western Blot).
Low intensity at the test line	Sample was below the working concentration range	Reduce dilution to 10-fold and retest using a new test strip.
	Sample was above the working concentration range	Perform a second dilution on the diluted sample to bring the concentration into the working concentration range.
No control line detected	Incorrect operation	Please repeat the test according to the instructions.
	Test card exceeds expiration date	Use the test card within the expiration date.

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