Flag-His-Tag Rapid Assay Kit

Cat No.	Size
RA10085	10T
RA10085	100T

Store at room temperature, shelf life: 1 year

1、Introduction

The Flag-His-Tag Rapid Assay Kit is a semi-quantitative protein detection reagent based on colloidal gold lateral flow chromatography technology. Its main structure consists of a sample pad, gold conjugate pad, chromatography membrane, absorbent pad, and backing plate. Goat anti-mouse IgG polyclonal antibody (Control Line) and mouse anti-Flag-Tag monoclonal antibody (Test Line) are immobilized on the chromatography membrane; colloidal gold-labeled mouse anti-His-Tag monoclonal antibody is immobilized on the gold conjugate pad.

When the test sample is added to the sample pad, the liquid moves toward the gold conjugate pad via capillary action. When the liquid containing Flag-His-Tag protein passes through the gold conjugate pad, it binds to the colloidal gold-labeled mouse anti-His-Tag monoclonal antibody. The complex migrates to the Test Line and is captured by the immobilized mouse anti-Flag-Tag monoclonal antibody. As the captured complex accumulates, a purplish-red band appears at the Test Line. When the complex moves to the Control Line, the goat anti-mouse IgG antibody captures the colloidal gold-labeled mouse monoclonal antibody, forming a purplish-red band at the Control Line. The presence of Flag-His-Tag protein in the sample is determined by reading whether the Test Line and Control Line on the chromatography membrane develop color.

The detection limit of this card is 4ng/mL. When the concentration of Flag-His-Tag protein in the test sample is between 4ng/mL and 500ng/mL, the color depth of the Test Line is positively correlated with the concentration of Flag-His-Tag protein in the sample. When the concentration of Flag-His-Tag protein exceeds 500ng/mL up to 10μg/mL, the Test Line intensity remains at a high level and does not show significant weakening with excessive analyte.

2、Intended Use

For rapid detection of Flag-His-Tag protein products obtained from prokaryotic and eukaryotic expression systems.

3、Components

1) Detection Card; 2) Running Buffer; 3) Instructions for Use

4、Usage Method

1. Open the package of the detection card and place it flat on the laboratory bench.
2. Pre-dilution of test samples:

   Proper pre-dilution of samples (to bring the target protein concentration within the working range of 4-

500ng/mL for this detection reagent) is critical to the accuracy of test results. Excessively high protein concentration (>500ng/mL) may cause weakening of the Test Line color or saturation of color intensity, leading to distorted protein concentration reading; excessively low protein concentration (<4ng/mL) may cause the Test Line color to be too faint to be read with the naked eye.

If the concentration of the target tag protein is known, directly dilute the sample to 100ng/mL using the reagent's accompanying chromatography buffer.

If the concentration is unknown, dilute samples from bacterial, mammalian, yeast, and insect cell lysates 50-fold using the reagent's accompanying chromatography buffer, then mix by vortexing.

3. Pipette 20μL of the diluted test specimen and add it to the sample well.
4. Add 50μL of chromatography buffer to the sample well (2 drops from a vertical dropper bottle), and read the results after 10-15 minutes.

Note: If the test result of the pre-diluted sample shows that the Test Line color is extremely faint, difficult to distinguish with the naked eye, or significantly lower than the expected concentration, there is a possibility that the sample concentration is too high (e.g., the sample concentration exceeds 1mg/mL). It is recommended to perform a second 100-fold dilution of the pre-diluted sample and repeat the test. If a visible band appears at the Test Line, it indicates that the initial result was indeed caused by excessively high protein concentration; if no visible band is observed, it indicates that your analyte does not contain Flag-His-Tag protein or its initial content is below 200ng/mL. If you suspect that low protein concentration causes the faint Test Line color, you can reduce the pre-dilution factor to 10-fold. We do not recommend a pre-dilution factor lower than 10-fold, as test results under such conditions are susceptible to interference from complex components in the protein solution, leading to reduced detection accuracy.

5、Compatibility Limits with Common Detergents and Salts:

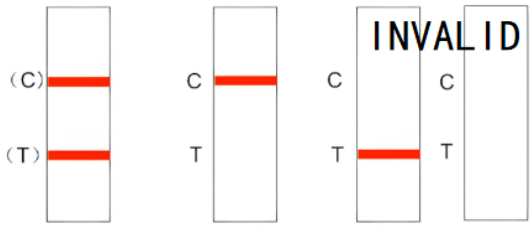
Name	Maximum Concentration
NaCl	1.5M
Urea	0.4M
TritonX-100	1%
Tween-20	1%
SDS	0.20%
NP-40	1%
EDTA	7.5mM
Glycerol	10%
KCl	1.5M
CHAPS	1.0%
RIPA	100%

6、Result Interpretation

1、Positive: Both the Control Line and Test Line develop color in the detection window.

2、Negative: The Control Line develops color, and the Test Line does not develop color.

3、Invalid: If the Control Line does not develop color, the test is invalid regardless of whether the Test Line develops color.



7、Troubleshooting

Phenomenon	Possible Cause	Solution
No visible band at the Test Line	The test sample does not contain Flag-His-Tag protein	Check whether the correct detection card corresponding to the test sample is used
		Verify the presence of the tag protein by ELISA or WB methods
Very faint color intensity of the Test Line	Sample concentration is lower than the lower limit of the working concentration range of the detection card	Reduce the sample dilution factor to 10-fold and retest
	Sample concentration is higher than the upper limit of the working concentration range of the detection card	Perform a second dilution of the sample to bring its concentration within the working range of the detection card
No visible band at the Control Line	Operational error	Repeat the test according to the instructions, and confirm that the concentrations of lysis and extraction reagents added to the sample are within the recommended ranges
	The detection card has expired	Use a detection card within the validity period