Flag-Tag Rapid Competitive Assay Kit

Cat No.	Size
RA10084	10T
RA10084	100T

Store at room temperature, shelf life: 1 year

1、Introduction

The Flag-Tag Rapid Competitive 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. A secondary antibody against the gold-labeled antibody (Control Line) and Flag-tagged protein (Test Line) are immobilized on the chromatography membrane; a colloidal gold-labeled anti-Flag-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. If the test sample does not contain Flag-Tag protein, the Flag-Tag gold-labeled antibody moves to the Test Line position with the laminar flow and specifically binds to the immobilized Flag-tagged protein, forming a purplish-red band at the Test Line. If the test sample contains Flag-Tag protein, the Flag-Tag gold-labeled antibody specifically binds to the Flag-Tag protein in the sample. The more Flag-Tag protein in the sample, the more gold-labeled Flag-Tag antibody binds to it, and the less free Flag-Tag gold-labeled antibody remains. When the free antibody moves to the Test Line position, it binds to the immobilized Flag-tagged protein, forming a faint purplish-red band or no band at all.

When the complex moves to the Control Line, the Flag-Tag gold-labeled antibody is captured by the secondary antibody against the gold-labeled antibody coated on the Control Line, forming a purplish-red band. The presence of Flag-Tag protein in the sample is determined by reading whether the Test Line and Control Line on the chromatography membrane develop color and the intensity of the color.

The detection limit of this card is 1μg/mL. That is, when the concentration of Flag-Tag protein in the test sample reaches 1μg/mL or higher, the intensity of the Test Line will be effectively weakened, showing a faint purplish-red band or no band. When the concentration of Flag-Tag protein in the test sample is between 1μg/mL and 10μg/mL, the color depth of the Test Line is negatively correlated with the concentration of Flag-Tag protein in the sample. When the concentration of Flag-Tag protein in the sample reaches 10μg/mL or higher, the Test Line disappears, i.e., no visible band appears to the naked eye.

2、Intended Use

For rapid detection of Flag-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:

   If the concentration of the target tag protein is known, directly dilute the sample to 3μg/mL using the reagent's accompanying chromatography buffer.

   If the concentration is unknown, dilute samples from bacterial lysates 25-fold and samples from mammalian cell supernatants or lysates 5-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: For recombinant tag proteins with low expression abundance, dilution may result in concentrations below the detection limit of this product, leading to false-negative results. In such cases, 20μL of undiluted sample stock can be added, followed by 50 μ L of accompanying chromatography dilution buffer to complete the chromatography process. However, it should be noted that in this case, the complex matrix in the sample may cause interference to the detection. It is particularly necessary to set up a negative control with the same matrix composition as the sample. Whether the tag protein is expressed can be determined by comparing the color intensity of the Test Line between the test sample and the negative control.

If the test result of the pre-diluted sample shows that the Test Line color is extremely faint and difficult to distinguish with the naked eye, there is a possibility that the sample concentration is too high (e.g., the concentration of the pre-diluted sample exceeds 10μg/mL). It is recommended to perform a second 10-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 faint color was indeed caused by excessively high protein concentration.

5、Compatibility Limits with Common Detergents and Salts:

Name	Maximum Concentration
NaCl	0.25M
Urea	0.4M
TritonX-100	1%
Tween-20	1%
SDS	0.20%
NP-40	1%
EDTA	5mM
Glycerol	10%
KCl	0.25M

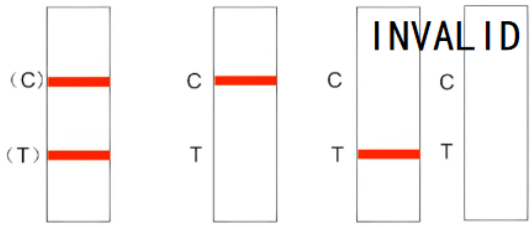
CHAPS	1.0%
RIPA	100%

6、Result Interpretation

1、Negative: Both the Control Line and Test Line develop color in the detection window, and the color intensity of the Test Line is consistent with that of the negative control.

2、Positive: The Control Line develops color, and the Test Line does not develop color or the color intensity of the Test Line is significantly weaker than that of the negative control.

3、Invalid: If the Control Line does not develop color, the test is invalid regardless of whether the Test Line develops color (as shown in the figure).



7、Troubleshooting

Phenomenon	Possible Cause	Solution
No visible color weakening of the Test Line compared to the negative control	The test sample does not contain Flag-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 higher than the upper limit of the working concentration range of the detection card	Perform a second dilution of the test sample and repeat the test
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