DYKDDDDK(Flag) Tag Antibody Plate

Technical Manual No. TM0640 Version 01262022

Product Name	Cat.No	Size
DYKDDDDK(Flag) Tag Antibody Plate (Clear, 96-well)	L00455C	5 plates
DYKDDDDK(Flag) Tag Antibody Plate (White, 96-well)	L00455W	5 plates
DYKDDDDK(Flag) Tag Antibody Plate (Black, 96-well)	L00455B	5 plates

The product is used for rapid capture of Flag-tagged protein in different samples.

The operator should read technical manual carefully before using this product.

For research use only. Not for use in diagnostic procedures.

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I. Description

Flag tag is a polypeptide of DYKDDDDK amino acid sequence that can be added to a target protein using recombinant DNA technology. It can be fused to N-terminus or C-terminus of the protein to facilitate detection and purification. Flag-tagged protein is usually expressed in *E. coli* and mammalian cells. Anti-Flag tag antibody is a useful tool for the analysis of Flag-tagged protein with different methods such as western blot, immunoprecipitation and flow cytometry.

DYKDDDDK(Flag) Tag Antibody Plate is a 96-well microtiter plate coated with THE™ Anti DYKDDDDK Tag Antibody. It can bind target proteins with high specificity and capacity. Its capacity and sensitivity varies, depending on protein size, structure and solution environment. Generally, the plate is more sensitive to capture Flag-tagged protein with lower molecular weight. Any detection reagent, which can recognize with mouse IgG, **should not be** applied for the plate, such as anti mouse IgG antibodies and protein A.

The product is developed for rapid capture of Flag-tagged protein in different samples, including Flag-tagged proteins from *Ecoli*, yeast and mammalian extracts and cell culture supernatant. The plate can be applied to many assays, from direct Flag-tagged protein detection and screening, to more comprehensive protein-protein interaction assays. There are several potential applications:

- Capture Flag-tagged protein or bio-molecules complexes containing Flag-tagged protein. It is a useful tool for high throughput immuno identification assay.
- Provides a platform to compare protein expression level in different culture condition, to determine target protein phosphorylation condition and to detect specific antibody in human and rat serum.
- Suitable for high throughput screening of stable cell lines expressing Flag-tagged protein or humanized antibody with Flag tag.

GenScript provides **clear**, **white** or **black** plates respectively to satisfy different assay demands, including colorimetric, chemiluminescent or fluorescent assay.

II. Key Features

Features	Specifications
Pre-coated Antibody	THE™ DYKDDDDK Tag Antibody, mAb, Mouse
Specificity	N-terminal/C-terminal/internal Flag-tagged protein
Sensitivity	1 ng/well
Capacity	100~300 ng/well
Reagents Compatibility	Compatible with common used reagents (see *Reagent Compatibility Tests* table)

III. Storage

DYKDDDDK(Flag) Tag Antibody Plate should be shipped on ice pack. The unopened plate is stable for at least 2 years when stored at 2-8 °C. The product is more stable when stored at -20 °C. The opened plate should be used within one week.

IV. Instruction for Use

- All the reagents should be equilibrated to room temperature (20-25 °C) before test.
- This manual gives general protocols for different assays. The user should optimize the protocol to achieve ideal test result.
- For protein expression screening or protein quantification assay, the user should choose proper DYKDDDDK(Flag) Tag Antibody Plate and corresponding detection reagent. For example, Biotin or HRP-conjugated antibody against target protein can be used for DYKDDDDK(Flag) Tag Antibody Plate (Clear, 96-well).
- For DYKDDDDK(Flag) Tag Antibody Plate (Clear, 96-well), Count the strips of DYKDDDDK(Flag) Tag Antibody Plate required for an assay and leave the unused strips in the foil pouch and store at 2-8 °C.

1. Immuno capture of Flag-tagged protein complex

- The sample volume can vary from 100~200 µl/well, depending on the amount of Flag-tagged protein in sample.

Materials and Equipments Required

- **Provided in the Kit**

  DYKDDDDK(Flag) Tag Antibody Plate

  Plate sealer

- **Not provided in the Kit**

  Test sample containing Flag-tagged proteins

  1 X Washing Solution (1X Phosphate-Buffered Saline, 0.1% Tween 20 Detergent (PBST))

SDS-PAGE Sample Buffer: 1X LDS Sample Buffer (GenScript Cat#M00676) with 50mM DTT (freshly added)

Pipettor

Microtube

Procedure Guideline

1.1 Add 100 µl of test samples containing Flag-tagged protein, negative control and positive control to each well of DYKDDDDK(Flag) Tag Antibody Plate.

1.2 Cover the plate with plate sealer and incubate at room temperature for 1~3 hours or incubate at 4 °C overnight.

1.3 Wash the plate with 260 µl/well of 1 X Washing Solution for four times.

1.4 Add 30 µl of SDS-PAGE Sample Buffer to each well and incubate for 10~15 minutes.

1.5 Pipette up and down the SDS-PAGE Sample Buffer several times and transfer to storage tubes. Denature samples at 95°C for 5 minutes.

1.6 Store the eluted sample at -20 °C or perform Western Blot analysis immediately.

2. Protein Expression Screening

- This procedure utilizes sandwich ELISA method to perform protein expression screening in samples.
- Other enzyme-substrate developing system besides HRP-TMB could also be used in this application.

Materials and Equipments Required

- **Provided in the Kit**

  DYKDDDDK(Flag) Tag Antibody Plate

  Plate sealer

- **Not provided in the Kit**

  Samples containing Flag-tagged protein

  HRP-conjugated antibody against target protein

  TMB Substrate

  Stop Solution (1M HCl or 1M H_2SO_4 or 1M H_3PO_4)

  1 X Washing Solution (1X Phosphate-Buffered Saline, 0.1% Tween 20 Detergent (PBST))

  Pipette

  Microplate reader capable of measuring absorbance at 450 nm

Procedure Guideline

2.1 Add 100 µl of test samples containing Flag-tagged protein, negative control or positive control to different wells of DYKDDDDK(Flag) Tag Antibody Plate.

2.2 Cover the plate with plate sealer and incubate at room temperature for 1~3 hours or at 4 °C

overnight.

2.3 Wash the plate with 260 µl/well of 1 X Washing Solution for four times.

2.4 Add 100 µl of prepared HRP-conjugated antibody against target protein to each well.

2.5 Cover the plate with plate sealer and incubate at room temperature for 1 hour.

2.6 Wash the plate with 260 µl/well of 1 X Washing Solution for four times.

2.7 Add 100 µl of TMB Substrate to each well and incubate at room temperature for 10~20 minutes, or keep close monitoring on the developing process until desired developing color observed.

2.8 Add 50 µl of Stop Solution to each well to stop the reaction.

2.9 Read absorbance of the plate on a microplate reader at 450 nm.

3. Protein Quantification

- This procedure utilizes sandwich ELISA method to quantify Flag-tagged proteins in samples.
- Before test, the researcher should do preliminary experiment to set up a standard curve of Flag-tagged protein of interest.
- Other enzyme-substrate developing system besides HRP-TMB could also be used in this application.

Materials and Equipments Required

- **Provided in the Kit**

  DYKDDDDK(Flag) Tag Antibody Plate

  Plate sealer

- **Not provided in the Kit**

  Samples containing Flag-tagged proteins

  Flag-tagged protein standard

  HRP-conjugated antibody against target protein

  TMB Substrate

  1 X Washing Solution (1X Phosphate-Buffered Saline, 0.1% Tween 20 Detergent (PBST))

  Stop Solution (1M HCl or 1M H_2SO_4 or 1M H_3PO_4)

  Pipettor

  Microplate reader capable of measuring absorbance at 450 nm

Procedure Guideline

3.1 Add 100 µl of prepared Flag-tagged protein standards and test samples into the plate.

3.2 Cover the plate with plate sealer and incubate the plate at room temperature for 1~3 hours or at 4 °C overnight.

3.3 Wash the plate with 260 µl/well of 1 X Washing Solution for four times.

3.4 Add 100 µl of the prepared HRP-conjugated antibody against target protein to all wells.

3.5 Cover the plate with plate sealer and incubate at room temperature for 1 hour.

3.6 Wash the plate with 260 µl/well of 1 X Washing Solution for four times.

3.7 Add 100 µl of TMB Substrate to each well and incubate at room temperature for 10~20 minutes, or keep close monitoring on the developing process until desired developing color observed.

3.8 Add 50 µl of Stop Solution to each well to stop the reaction.

3.9 Read absorbance of the plate on a microplate reader at 450 nm.

3.10 Generate a standard curve by plotting the average absorbance on the Y axis versus the corresponding Flag tag protein standard concentration on the X axis.

3.11 The amount of Flag-tagged protein in each sample is determined by extrapolating OD values to the standard curve.

V. Reagent Compatibility

Some reagents may interfere with the test results. Check the reagents concentration according to the Reagent Compatibility Tests table. Dialyse or dilute samples if needed.

Substance	Compatible Concentration
Triton X-100	< 5%
Guanidine HCl	< 0.05 mM
Urea	< 0.5 M
Deoxycholic Acid	< 0.05%
SDS	< 0.03%
DTT	< 10 mM
β-ME	< 10 mM
Tween-20	< 5%
CHAPS	< 0.1%
NaCl	< 1M
TBS	Compatible
PBS	Compatible
RIPA Lysis Buffer	Compatible

VI. Troubleshooting

Aassay	Problem	Probable Cause	Solution
Immuno Capture of Flag-tagged protein complex	No signal in Western blot assay	Flag-tagged proteins are not expressed.	Optimizing expression condition and prepare new sample
		Flag-tagged proteins are not captured on the plate	Check Reagent Compatibility table, to optimize reagent concentration
			Increasing incubation time with sample
			Check sample pH value. The binding performs well in neutral (pH6.8-7.4) condition.
Protein expression screening or protein quantitation	No signal	HRP-conjugated antibody against target protein and Flag Tag antibody do not pair	Choose another HRP-conjugated antibody against target protein as alternative
	Weak signal	Flag-tagged protein is low in sample	Increase incubation time with sample
			Concentrate sample
		Over diluted HRP-conjugated antibody against target protein	Increase HRP-conjugated antibody concentration
		Antibody incubation time is short	Increase antibody incubation time
		Substrate incubation time is short	Increase substrate incubation time
		Incompatible reagent(s) concentration	Check and decrease concentration of reagent(s) in sample
	High Background	High concentration of HRP-conjugated antibody against target protein	Dilute HRP-conjugated antibody against target protein
		Insufficient washing	Increase washing times
		Washing Solution is polluted	Use new prepared Washing Solution
		Substrate incubation time is too long	Decrease substrate incubation time

VII. Related Products

- Anti-DYKDDDDK IP Resin L00425
- Anti-DYKDDDDK G1 Affinity Resin L00432
- THE™ DYKDDDDK Tag Antibody, mAb, Mouse A00187
- THE™ DYKDDDDK Tag Antibody [HRP], mAb, Mouse A01428
- THE™ DYKDDDDK Tag Antibody [Biotin], mAb, Mouse A01429
- THE™ DYKDDDDK Tag Antibody [FITC], mAb, Mouse A01632
- DYKDDDDK-tag Antibody, pAb, Rabbit A00170
- DYKDDDDK Peptide RP10586
- DYKDDDDK Lysates M0005

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