ABSbio™ DYKDDDDK tag ELISA Kit (SE002-flag)

Flag-tagged protein Quantification

Introduction

Epitope tags provide a convenient way to isolate interacting proteins without the need for specific antibodies to each new protein. The FLAG can be fused to N- or C-terminus of the protein and has been used in expression systems for the detection, quantification and purification of heterologous proteins in many biological systems including *E.coli* and mammalian systems. 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.

ABSbio™ DYKDDDDK (flag) tag ELISA Kit provide a convenient, ready-to-use, high-throughput platform for rapid capture and detection of recombinant flag fusion proteins in different samples. The High Sensitivity plate is coated with the mouse monoclonal antibody (Binds to the same epitope as Sigma 'Anti-FLAG' M2 Antibody, Anti-FLAG is a trademark of Sigma-Aldrich) and pre-blocked to provide timesaving for high-throughput users. The monoclonal antibody is covalently bound to the plate in a favorable orientation such that the Fab region of the antibody is available for the DYKDDDDK (flag) tag to provide greater specificity. The anti-DYKDDDDK high sensitivity antibody coated multiwell plate can be used to detect N-terminal, Met-N-terminal, internal, and C-terminal flag and 3xflag fusion proteins. The kit can detect as little as 1 ng/ml with a capacity of up to 300 ng/well of flag fusion protein.

The kit uses a double-antibody sandwich enzyme-linked immunosorbent assay (ELISA) to analyze the level of DYKDDDDK -fusion protein in samples. First add standard and sample to wells pre-coated with anti-DYKDDDDK antibody to capture available flag-fusion protein in solution, and perform incubating and washing procedures to remove unbound substance. Then add detection antibody to bind the captured flag-fusion protein, followed by another round of incubation and washing procedures to remove unbound antibody-HRP. Finally, TMB substrates are added, incubated for detection, and a blue color is developed. Reaction is stopped and color turns to yellow when Stopping Solution (acidic) is added. The yellow color intensity proportionally correlates to the concentration of the flag-fusion protein in samples.

Kit Components (96 tests)

flag-fusion protein Standard:	3 vials	Assay Diluent:	50 mL	TMB Solution:	12 mL	Plate sealer:	2
Detection Ab:	60 µL	Wash Solution:	50 mL	Stop Solution:	12 mL	Anti-flag antibody coated plate:	1

Storage and Handling: Shipping on ice. Store Standard and Detection Ab at -20°C, other components at 2-8 °C. Shelf Life: 3 months after receipt. Warm up reagents and plate to room temperature before use, keep Standard and Detection Ab on ice.

Protein Expression Screening & Quantification

- This procedure utilizes sandwich ELISA method to perform protein expression screening & quantify in samples.
- **Wash Solution:** 10x dilute Wash Solution with dH_2O to prepare 1x Wash Solution.
- **Flag-fusion Protein Standard (3 vials):** Each vial contains 12 µl of the standard sufficient for a 96-well plate. The undiluted standard can be stored at -20° C for up to 3 months if not used immediately. Spin to bring down the material prior to open the tube. Add 10 µl of the standard to 490 µl of Assay Diluent to make the high standard concentration of 200 ng /ml. Vortex briefly and allow it to sit for a minimum of 15 min prior to use. A seven point standard curve is generated using 2-fold serial dilutions in Assay Diluent, vortex briefly for each of dilution step. Store the rest of the standard at -20° C.
- **Detection Ab:** Immediately before use, add 50 µl of the antibody into 11 mL of Assay Diluent for one plate (for partial plate assay, adjust the volumes accordingly. Store the rest of the antibody at -20° C).

Procedure Guideline

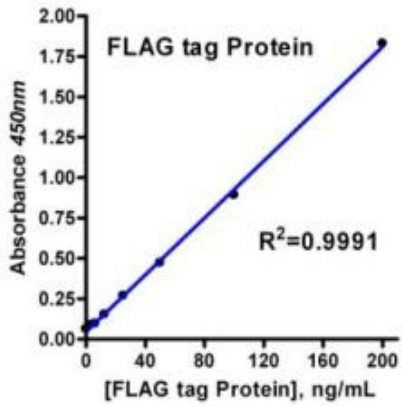
1. Prepare sample dilutions (10 fold series dilution) in a clean 96-well plate with 1x PBS.
2. Set standard wells, testing sample and blank wells on the assay plate/strip. Transfer diluted standard 100 µl to standard wells, diluted sample 100 µl to sample wells, 100 µl sample diluent only to blank wells. Assay should be run in duplicate.

	1	2	3	4	5	6	7	8	9	10	11	12
A	200ng/mL standard	200ng/mL standard	sample1 (1:1)	sample1 (1:1)	Sample2 (1:1)	Sample2 (1:1)						
B	100ng/mL	100ng/mL	sample1 (1:10)	sample1 (1:10)	Sample2 (1:10)	Sample2 (1:10)						
C	50ng/mL	50ng/mL	sample1 (1:100)	sample1 (1:100)	Sample2 (1:100)	Sample2 (1:100)						
D	25ng/mL	25ng/mL	sample1 (1:1000)	sample1 (1:1000)	Sample2 (1:1000)	Sample2 (1:1000)						
E	12.5ng/mL	12.5ng/mL	sample1 (1:10k)	sample1 (1:10k)	Sample2 (1:10k)	Sample2 (1:10k)						
F	6.25ng/mL	6.25ng/mL	s sample1 (1:100k)	s sample1 (1:100k)	s sample2 (1:100k)	s sample2 (1:100k)						
G	3.13ng/mL	3.13ng/mL	sample1 (1:1000k)	sample1 (1:1000k)	Sample2 (1:1000k)	Sample2 (1:1000k)						
H	blank	Blank	sample1 (1:10000k)	sample1 (1:10000k)	Sample2 (1:10000k)	Sample2 (1:10000k)						

3. Cover the plate with plate sealer and incubate the plate at room temperature for 2 hrs or at 37 °C for 1h.
4. Decant as much liquid as possible, fill the wells with 250 µl wash solution, oscillate the plate on an oscillating shaker if available for 1 min, decant the wash solution and remove residual liquid with absorbent paper. Repeat wash three times.

5. Add 100 µl of the prepared detection antibody to all wells.
6. Cover the plate with plate sealer and incubate at room temperature for 1 hour.
7. Wash the plate or strips as described in Step#4.
8. Add 100 µl of TMB Solution to each well and incubate at room temperature for 10~30 minutes, or keep close monitoring on the developing process until desired developing blue color observed.
9. Add 100 µl of Stop Solution to each well to stop the reaction (the blue color change to yellow).
10. Read absorbance of the plate on a microplate reader at 450 nm within 15 min.
11. Average the duplicate OD readings for each standard and sample. Create a standard curve by subtracting the mean OD's for the blank. Construct standard curve (plotting the mean OD450 for each standard on the X-axis against concentration on the Y-axis, draw a best-fit curve through the points) and calculate linear regression equation, then use sample OD values and regression equation to calculate the corresponding sample concentration. It should be remembered that the sample has been diluted and its actual concentration should be justified by dilution factor (the measurement and calculation can be accomplished by software like SoftMax).
12. If molecular weight of sample differs from protein standard (MW 10kD) apply the following equation to the reading concentration to obtain the actual concentration**= [MW Sample] / [MW protein standard] x Sample reading (ng/mL).**

Typical Standard Curve



Always run your own standard curves for calculation of results.

Quantification of flag-tagged Protein.

FLAG-tag protein was serially diluted and then incubated at room temperature for 2 hours in the anti-DYKDDDDK coated plate. For ELISA analysis using DYKDDDDK (flag)-tag ELISA Kit (#SE002-flag), the FLAG-tag protein was incubated with detection antibody for 1 hour at room temperature. Following four wash steps. The peroxidase was detected by using a soluble TMB solution for ELISA applications. The reaction was stopped using stop solution. Absorbance was read on a Molecular Devices SpectraMax® at 450 nm.

Related Products:

DYKDDDDK **(flag)-tag ELISA Kit (#SE003-flag)**

DYKDDDDK**(flag)-tag Antibody Coated Plate (#SE001-flag)**

TROUBLE SHOOTING GUIDE

1. To obtain reliable and reproducible results the operator should carefully read this manual and fully understand all aspects of each assay step before attempting to run the assay.
2. Throughout the assay the operator should adhere strictly to the procedures with good laboratory practice.
3. Have all necessary reagents and equipment ready on hand before starting. Once the assay has been started all steps should be completed with precise timing and without interruption.
4. Avoid cross contamination of any reagents or samples to be used in the assay.
5. Make sure that all reagents and samples are added to the bottom of each well.
6. Careful and complete mixing of solutions in the well is critical. Poor assay precision will result from incomplete mixing or cross well contamination due to inappropriate mixing.
7. Remove any air bubble formed in the well after acidification of substrate solution because bubbles interfere with spectrophotometric readings.
8. High absorbance in background or blank wells could be due to 1) cross well contamination by standard solution or sample or 2) inadequate washing of wells with washing solution.