ab285234 – DYKDDDDK-Tag Protein ELISA Kit

For the In vitro, quantitative determination of DYKDDDDK-tag fusion proteins in E. coli and mammalian cells.
For research use only - not intended for diagnostic use.
PLEASE NOTE: With the acquisition of BioVision by Abcam, we have made some changes to component names and packaging to better align with our global standards as we work towards environmental-friendly and efficient growth. You are receiving the same high-quality products as always, with no changes to specifications or protocols.

For overview, typical data and additional information please visit:
http://www.abcam.com/ab285234

Introduction

DYKDDDDK tag has a small unique protein sequence that is commonly fused to N- or C-terminus of a protein for expression. Expression and detection of DYKDDDDK-tag proteins are frequently performed in many academic and industry laboratories because this tag is generally more hydrophilic than other common tags and therefore it is less likely to denature or inactivate proteins to which it is attached. In addition, DYKDDDDK-tag fusion proteins in *E. coli* or mammalian cell lysate can be easily purified by affinity chromatography using specific resins. Typically, DYKDDDDK-tag proteins are detected by SDS-PAGE or western blots, but these approaches are generally more laborious, time-consuming, and less sensitive. This DYKDDDDK-Tag Protein ELISA Kit (ab285234, E4700) is based on the competitive ELISA principle, and it is an easy, fast, and sensitive method to detect the expressed DYKDDDDK-tag proteins. This detection kit offers ready-to-use reagents, and can detect as low as 5 nM of DYKDDDDK-tag protein in approximately 1.5 hrs.

Applications

In vitro, quantitative determination of DYKDDDDK-tag fusion proteins
Detection Range: 5-500 nM
Sensitivity: 5 nM

Storage and Stability

The entire kit may be stored at -20°C for up to 12 months from the date of shipment. Opened kit is stable for 1 month at -20°C.

Materials Supplied

Item	Quantity	Storage Condition
DYKDDDDK Microplate/ELISA Microplate	8 X 12 Strips	-20°C
DYKDDDDK Standard	1 vial	-20°C
Goat Anti-Rabbit HRP Conjugate I/HRP-conjugate Stock	25 µL	-20°C
DYKDDDDK Antibody/Antibody	7 ml	-20°C
TMB Substrate I/TMB substrate	10 ml	-20°C
Stop Solution VIII/Stop Solution	10 ml	-20°C
10X Wash Buffer II/Wash Buffer (10X)	50 ml	-20°C
Conjugate Buffer III/Conjugate Buffer	7.5 ml	-20°C
Microplate Sealing Film/Plate Sealers	4	-20°C

Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully utilize this assay:

- Microplate reader capable of measuring absorbance at 450 and 650 nm
- Clean Eppendorf tubes for preparing standards and sample dilutions.

Reagent Preparation

- Bring all reagents to room temperature before use.
- Before using the kit, spin tubes and bring down all components to the bottom of tubes.

TMB Substrate I/TMB Substrate and Stop Solution VIII/Stop Solution: Ready to be used. After use, store them at 4°C.

10X Wash Buffer II/Wash Buffer (10X): Bring bottle to room temperature. If crystals are present, warm up to room temperature and mix gently until the crystals are completely dissolved. Prepare 100 ml of 1X Wash Buffer II/Wash Buffer by diluting 10 ml of 10X Wash Buffer II/Wash Buffer (10X) with 90 ml deionized water. The 1X solution can be stable at 4°C for one month.

HRP-conjugate working solution: Spin briefly before opening the tube. Pipet 12 µl of Goat Anti-Rabbit HRP Conjugate I/HRP-conjugate Stock into Conjugate Buffer III/Conjugate Buffer (7.5 ml) bottle to prepare conjugate working solution. Vortex the conjugate solution bottle for a minute. The conjugate working solution is stable at 4°C for 2 months.

Standard Preparation

1. DYKDDDDK Standard: Add 510 µl of deionized water into the vial to prepare 500 nM (S6).
2. Perform 2.5-fold serial dilutions from S6 (e.g. 40 µl mixed with 60 µl of water) to prepare S5 to S1 standards sequentially.
   S0 contains water only.
3. Keep the prepared standards on ice during the assay.
4. The diluted standards should be stable at -20°C for 2 weeks. (Avoid freeze-thaw cycles)

Standards	S0	S1	S2	S3	S4	S5	S6
Concentrations (nM)	0	5.12	12.8	32.8	80	200	500

Sample Preparation

- Always prepare a control without DYKDDDDK-tag protein expression (empty vector) to subtract background.
- The dilution factors are recommended below but may be varied depending on your sample concentration.

E. coli: Spin 5 ml of E. coli cells (OD at 600 nm > 1.5) in a centrifugation tube at 10,000 x g for 2 min. Discard the medium and collect the pellet. Add ~3 ml of PBS and vortex to disperse the pellet. Lyse the cells by sonication for 2 min and then spin the cells at 10,000 g and 4°C for 15 min. Discard the pellet and collect the supernatant. Dilute the supernatant by 20 folds in water (e.g., 10 µl in 190 µl of water). Use 50 µl per well for the assay.
Δ Note: Dilution factor: 20

Mammalian Cells: Spin 2-3 ml of mammalian cells (10^5 – 10^6 cells/ml) in a centrifugation at 10,000xg for 2 min. Collect the pellet and discard the supernatant. Add 0.5-1 ml of 1X PBS into the tube and vortex to disperse the cell pellet. Lyse the cells by sonication for 2 min on ice and then spin the cells at 10,000 x g for 10 min. Collect the supernatant. Dilute the supernatant by 20 folds (e.g., 10 µl in 190 µl of water). Use 50 µl per well for the assay.
Δ Note: Dilution factor: 20

Assay Protocol

- It is recommended that all samples should be run in duplicate.
- Standard curves must be run each time an assay is performed.

1. Prepare all reagents, standards and samples as instructed.
2. Add 50 µl of Standards or Samples per well. Then add 50 µl of conjugate working solution and 50 µl of DYKDDDDK Antibody/Antibody to above wells.
3. Cover the plate with a plate sealer and mix well. Incubate the plate at room temperature (25°C) for 60 min.
4. Aspirate all reagents and wash each well 5 times: add 250 µl of 1X Wash Buffer II/Wash Buffer and incubate for 30 seconds. Remove 1X Wash Buffer II/Wash buffer completely before the next wash – this is essential for accurate results. Repeat this step 4 more times.
5. Add 100 µl of TMB Substrate I/TMB Substrate to each well. Tap or shake the plate to ensure complete mixing.
6. Check the OD at 650 nm for the well containing no DYKDDDDK std (S0). When its reading is between 0.8 and 1.0 (usually between 10-30 min after adding the TMB Substrate I/TMB Substrate), add 50 µl of Stop Solution VIII/Stop Solution and gently tap the plate to ensure thorough mixing.
7. Measure the OD at 450 nm immediately

Calculation:

The Standard Curve is done by plotting OD 450 nm of each standard solution (Y) vs. the respective concentration of the standard solution (X). The concentration of DYKDDDDK-tag protein in each sample (nM) can be interpolated from the standard curve. If the samples measured were diluted, multiply the dilution factor to the concentrations from interpolation to obtain the concentration before dilution.

Technical Support

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