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DYKDDDDK-Tag Protein ELISA Kit

(Catalog # E4700-100; 96 assays, Store kit at -20°C)

I. 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. BioVision's DYKDDDDK-Tag Protein ELISA Kit 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 100 ng of DYKDDDDK-tag protein in approximately 1.5 hrs.

II. Applications:

In vitro, quantitative determination of DYKDDDDK-tag fusion proteins
Detection Range: 256 – 10,000 ng/ml
Sensitivity: 100 ng/ml

III. Sample Type:

E. coli and mammalian cell lysates

IV. Kit Contents:

Components	E4700-100	Cap Code	Part Number
ELISA Microplate	8 X 12 Strips	--	E4700-100-1
DYKDDDDK Standard	1 vial	Yellow	E4700-100-2
HRP-conjugate Stock	25 µl	Blue	E4700-100-3
Antibody	7 ml	NM/Red	E4700-100-4
TMB substrate	10 ml	Amber	E4700-100-5
Stop Solution	10 ml	NM/Blue	E4700-100-6
Wash Buffer (10X)	50 ml	NM	E4700-100-7
Conjugate Buffer	7.5 ml	NM/Green	E4700-100-8
Plate Sealers	4	--	E4700-100-9

V. User Supplied Reagents and Equipment:

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

VI. Storage and Handling:

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.

VII. Reagent and Standard 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 and Stop Solution:** Ready to be used. After use, store them at 4°C.
- **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 by diluting 10 ml of 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 HRP-conjugate Stock into 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.
- **DYKDDDDK Standard**: Add 0.5 ml of deionized water into the vial to prepare 10,000 ng/ml (S5). Perform 2.5 fold serial dilutions from S5 (e.g. 40 µl mixed with 60 µl of water) to prepare S4 to S1 standards sequentially. S0 contains water only. Keep the prepared standards on ice during assay. The diluted standards should be stable at -20°C for 2 weeks. (avoid freeze-thaw cycles)

Standards	S0	S1	S2	S3	S4	S5
Concentrations (ppb)	0	256	640	1,600	4,000	10,000

VIII. Sample Preparation:

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

- ***E. coli***

1. 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.
2. Discard the medium and collect the pellet.
3. Add ~3 ml of PBS and vortex to disperse the pellet.
4. Lyse the cells by sonication for 2 min and then spin the cells at 10,000 g and 4°C for 15 min.
5. Discard the pellet and collect the supernatant.
6. Dilute the supernatant by 20 folds in water (eg. 10 µl in 190 µl of water).
7. Use 50 µl per well for the assay.

Note: Dilution factor: 20

Mammalian Cells

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

Note: Dilution factor: 20

IX. DYKDDDDK-Tag Protein ELISA Assay Protocol:

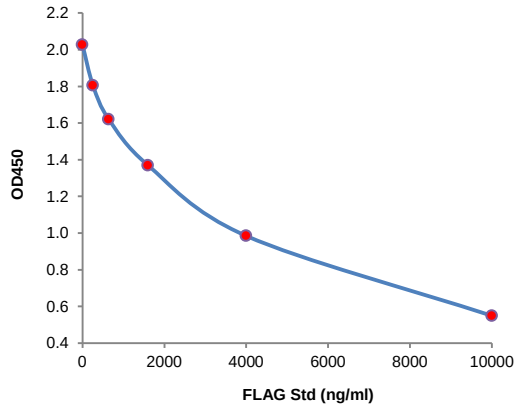
Notes: It is recommended that all samples should be run in duplicate. Standard curves must be run each time an assay is performed.

1. Prepare all reagents, standards and samples as sections VII and VIII respectively.
2. Add 50 µl of Standards or Samples per well. Then add 50 µl of conjugate working solution and 50 µl of 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 and incubate for 30 seconds. Remove 1X 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 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), add 50 µl of Stop Solution and gently tap the plate to ensure thorough mixing.
7. Measure the OD at 450 nm immediately.

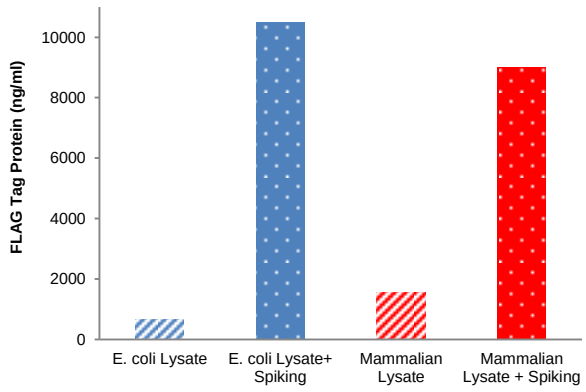
X. Calculation:

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 (ng/ml) 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.

A.



B.



Figures. **A.** Standard curve for DYKDDDDK-Tag ELISA Kit (*This standard curve is for demonstration only. A standard curve must be run with each assay*). **B.** Spike recovery experiment: *E. coli* and mammalian cell lysates were spiked with 9.3 ug/ml and 8.5 ug/ml of DYKDDDDK-tag protein and showed 88-100% recovery.

XI. RELATED PRODUCTS:

His-Tag Protein ELISA Kit (Cat. No. E4550-100)
Gentamicin (serum/urine) ELISA Kit (Cat. No. K4315-100)
Ampicillin ELISA Kit (Cat. No. E4350-100)
Enrofloxacin (ENR) ELISA Kit (Cat. No. E4277-100)
Fluoroquinolones ELISA Kit (Cat. No. K4205-100)
Vancomycin ELISA Kit (Cat. No. E4605-100)
Folic Acid ELISA Kit (Cat. No. E4523-100)
Kanamycin ELISA Kit (Cat. No. K4210-100)
Quinolone ELISA Kit (Cat. No. E4530-100)
Caffeine ELISA Kit (Cat. No. E4558-100)

FOR RESEARCH USE ONLY! Not to be used on humans.