Product Manual

DYKDDDDK-Tag Protein ELISA Kit

Catalog Numbers

AKR-5188	96 assays

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures

Introduction

DYKDDDDK is a short hydrophilic protein tag commonly used in conjunction with antibodies in protein pull-downs to study protein-protein interactions. The DYKDDDDK tag may be inserted at the N terminus, the N terminus preceded by a methionine residue, the C terminus, or internal positions of the target protein. The DYKDDDDK tag is commonly found on the surface of a fusion protein, which makes it more available as an epitope for binding to antibodies. The high hydrophilicity and small size of the DYKDDDDK tag tend to interfere less with protein expression, proteolytic maturation, antigenicity, and function. DYKDDDDK tags are also easily removed by enterokinase (EK).

Cell Biolabs' DYKDDDDK-Tag Protein ELISA Kit is a competitive enzyme immunoassay for rapid detection and quantitation of DYKDDDDK-tagged protein samples. The quantity of DYKDDDDK-tag in a protein sample is determined by comparing its absorbance with that of a known DYKDDDDK-tag protein standard. The kit has detection sensitivity range of 5 µg/mL to 78 ng/mL DYKDDDDK-tag GST protein standard, or 180 nM - 2.8 nM DYKDDDDK-tag residues. Each kit provides sufficient reagents for up to 96 assays including standard curve and DYKDDDDK-tag samples.

Assay Principle

The unknown DYKDDDDK-tag samples or recombinant DYKDDDDK-tag GST protein standards are first added to a DYKDDDDK conjugate coated plate. After a brief incubation, an anti-DYKDDDDK monoclonal antibody is added, followed by an HRP conjugated secondary antibody. The DYKDDDDK-tag protein content in unknown samples is determined by comparing with a standard curve that is prepared from predetermined DYKDDDDK-tag GST protein standards.

Related Products

1. AKR-121: GFP ELISA Kit
2. AKR-130: His Tag Protein ELISA Kit
3. AKR-5185: GST ELISA Kit
4. AKR-5186: Streptavidin ELISA Kit
5. AKR-5186: Avidin ELISA Kit

Kit Components

Box 1 (shipped at room temperature)

1. DYKDDDDK Conjugate Coated Plate (Part No. 51881B): One strip well 96-well plate
2. Anti-DYKDDDDK Monoclonal Antibody (Part No. 51882C): One 10 µL vial
3. Secondary Antibody, HRP Conjugate (1000X) (Part No. 10902): One 50 µL tube.
4. Assay Diluent (Part No. 310804): One 50 mL bottle
5. 10X Wash Buffer (Part No. 310806): One 100 mL bottle
6. Substrate Solution (Part No. 310807): One 12 mL amber bottle
7. Stop Solution (Part. No. 310808): One 12 mL bottle

Box 2 (shipped on blue ice packs)

8. Recombinant DYKDDDDK-Tag GST Protein Standard (Part No. 51883D): One 100 µL vial of 100 µg/mL recombinant, C-terminal DYKDDDDK-tag GST (28 kDa) in PBS containing BSA.

Materials Not Supplied

1. DYKDDDDK-Tag Protein Sample: purified or unpurified sample (cell or tissue lysate)
2. Microplate reader capable of reading at 450 nm (620 nm as optional reference wave length)

Storage

Upon receiving, store the DYKDDDDK-Tag GST Protein Standard at -20ºC. Store all other components at 4ºC.

Preparation of Reagents

- 1X Wash Buffer: Dilute the 10X Wash Buffer Concentrate to 1X with deionized water. Stir to homogeneity.
- Anti-DYKDDDDK Monoclonal Antibody: Immediately before use dilute the Anti-DYKDDDDK Monoclonal Antibody 1:1000 with Assay Diluent. Do not store diluted solutions.
- Secondary Antibody, HRP Conjugate: Immediately before use dilute the Secondary Antibody, HRP Conjugate 1:1000 with Assay Diluent. Do not store diluted solutions.

Preparation of DYKDDDDK-Tag Protein Standards

Centrifuge the Recombinant DYKDDDDK-Tag GST Protein Standard tube and mix well by pipetting up and down. Freshly prepare a dilution series of DYKDDDDK-Tag GST Protein Standard in the concentration range of 5 µg/mL – 78 ng/mL by diluting the DYKDDDDK-Tag GST Protein Standard stock solution in Assay Diluent (Table 1) or desired compatible lysis buffer.

Standard Tubes	Recombinant DYKDDDDK-Tag GST Protein Standard (µL)	Assay Diluent or Desired Lysis Buffer (µL)	DYKDDDDK-Tag Protein Standard Concentration (µg/mL)	DYKDDDDK-Tag Residue Concentration (nM)
1	20	380	5	180
2	200 of Tube #1	200	2.5	90
3	200 of Tube #2	200	1.25	45
4	200 of Tube #3	200	0.625	22.5
5	200 of Tube #4	200	0.313	11.3
6	200 of Tube #5	200	0.156	5.6
7	200 of Tube #6	200	0.078	2.8
8	0	200	0	0

Table 1. Preparation of DYKDDDDK-Tag GST Protein Standard Curve.
Note: Protein standards should be diluted in the same buffer as prepared samples.

Assay Protocol

Note: If testing mouse or rat plasma or serum, the IgG must be completely removed from each sample prior to testing, such as with Protein A or G beads. Additionally, a control well without primary antibody should be run for each sample to determine background signal.

1. Prepare and mix all reagents thoroughly before use.
2. Each DYKDDDDK-tag sample, DYKDDDDK-tag GST protein standard, and blank should be assayed in duplicate.
3. Add 50 µL of DYKDDDDK-tag sample or DYKDDDDK-tag GST protein standard to the DYKDDDDK Conjugate Coated Plate. Incubate at room temperature for 10 minutes on an orbital shaker.
4. Add 50 µL of diluted Anti-DYKDDDDK Monoclonal Antibody (see Preparation of Reagents Section) to each tested well.
5. Incubate at room temperature for 1 hour on an orbital shaker.
6. Wash microwell strips 5 times with 250 µL 1X Wash Buffer per well with thorough aspiration between each wash. After the last wash, empty wells and tap microwell strips on absorbent pad or paper towel to remove excess 1X Wash Buffer.
7. Add 100 µL of the diluted Secondary Antibody, HRP Conjugate (see Preparation of Reagents Section) to each tested well.
8. Incubate at room temperature for 1 hour on an orbital shaker.
9. Wash the strip wells 5 times according to step 6 above.
10. Warm Substrate Solution to room temperature. Add 100 µL of Substrate Solution to each well, including the blank wells. Incubate at room temperature for 2-30 minutes on an orbital shaker.

    Important Note: Watch plate carefully; if color changes rapidly, the reaction may need to be stopped sooner to prevent saturation.
11. Stop the enzyme reaction by adding 100 µL of Stop Solution into each well, including the blank wells. Results should be read immediately (color will fade over time).
12. Read absorbance of each microwell on a spectrophotometer using 450 nm as the primary wave length.

Example of Results

The following figures demonstrate typical DYKDDDDK-Tag Protein ELISA Kit results. One should use the data below for reference only. This data should not be used to interpret actual results.

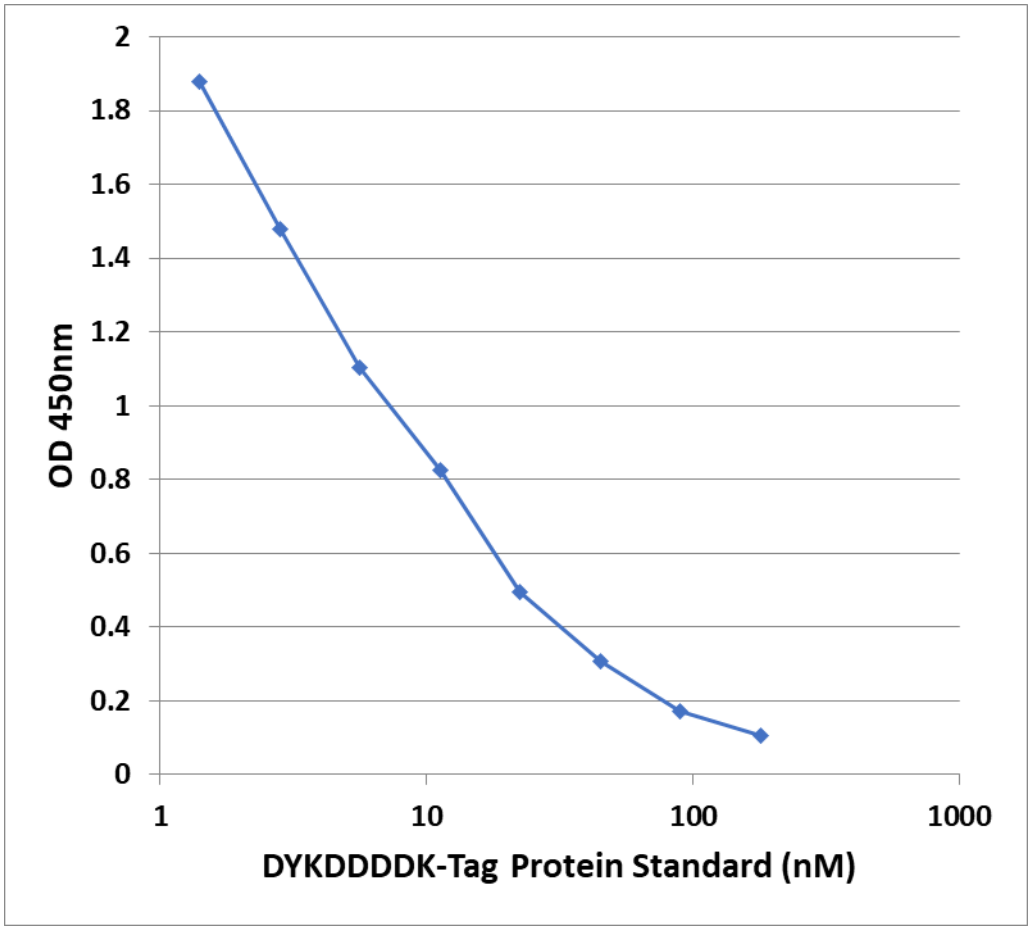

Figure 1: DYKDDDDK-Tag Protein Standard Curve. The Recombinant C terminal DYKDDDDK-Tagged GST was diluted in Assay Diluent and assayed as described in the Assay Protocol.

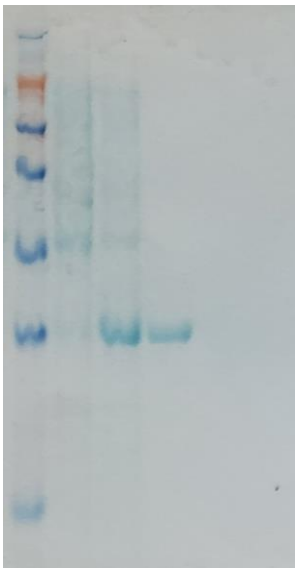

Figure 2: Purification of GST-DYKDDDDK Protein Standard. Lane 1: MW STDs; Lane 2: E.Coli Whole Lysate (-IPTG); Lane 3: E.Coli Whole Lysate (+IPTG); Lane 4: Elution Fraction for DYKDDDDK tag GST Protein Standard.

Appendix

C-terminal DYKDDDDK tag GST Protein Sequence: The DYKDDDDK tag is underlined.

MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFELGLEFPNLPYYIDGDV
KLTQSMAIIRYIADKHNMLGGCPKERAEISMLEGAVLDIRYGVSRIAYSKDFETLKVDFLSKLP
EMLKMFEDRLCHKTYLNGDHVTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPQI
DKYLKSSKYIAWPLQGWQATFGGGDHPPKSDLVPRGSDYKDDDDKRPHRD-

References

1. Terpe K. (2003) *Applied Microbiology and Biotechnology* **60**:523–533.
2. Hopp TP, Prickett KS, Price VL, et al. (1988) *Biotechnology* **6**:1204–1210.
3. Einhauer A, Jungbauer A. (2001) *Journal of Biochemical and Biophysical Methods* **49**:455–465.
4. Futatsumori-Sugai M, Abe R, Watanabe M, et al. (2009) *Protein Expression and Purification* **67**:148–155.

Warranty

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