DYKDDDDK-Tag Detection ELISA Kit

Item No. 501560

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GENERAL INFORMATION

Materials Supplied

Item Number	Item	96 wells Quantity/Size
401562	DYKDDDDK ELISA Monoclonal Antibody	1 vial/100 dtn
401564	DYKDDDDK-Protein ELISA Standard	1 vial/50 µg
401596	DYKDDDDK-HRP Tracer	1 vial/1 ml
401703	Immunoassay Buffer C Concentrate (10X)	1 vial/10 ml
400062	Wash Buffer Concentrate (400X)	1 vial/5 ml
400074	TMB Substrate Solution	2 vials/12 ml
10011355	HRP Stop Solution	1 vial/12 ml
400035	Polysorbate 20	1 vial/3 ml
400009	Precoated (Goat Anti-Mouse IgG) ELISA 96-Well Strip Plate	1 plate
400012	96-Well Cover Sheet	1 ea

If any of the items listed above are damaged or missing, please contact our Customer Service department at (800) 364-9897 or (734) 971-3335. We cannot accept any returns without prior authorization.

WARNING: THIS PRODUCT IS FOR RESEARCH ONLY - NOT FOR HUMAN OR VETERINARY DIAGNOSTIC OR THERAPEUTIC USE.

Safety Data

This material should be considered hazardous until further information becomes available. Do not ingest, inhale, get in eyes, on skin, or on clothing. Wash thoroughly after handling. Before use, the user must review the complete Safety Data Sheet, which has been sent *via* email to your institution.

Precautions

Please read these instructions carefully before beginning this assay.

This kit may not perform as described if any reagent or procedure is replaced or modified. The Stop Solution provided with this kit is an acid solution. Please wear appropriate personal protection equipment (*e.g.*, safety glasses, gloves, and lab coat) when using this material.

If You Have Problems

Technical Service Contact Information

Phone: 888-526-5351 (USA and Canada only) or 734-975-3888

E-Mail: techserv@caymanchem.com

In order for our staff to assist you quickly and efficiently, please be ready to supply the lot number of the kit (found on the outside of the box).

Storage and Stability

This kit will perform as specified if stored as directed at -20°C and used before the expiration date indicated on the outside of the box.

Materials Needed But Not Supplied

1. A plate reader capable of measuring absorbance at 450 nm
2. Adjustable pipettes; multichannel or repeating pipettor recommended
3. A source of ultrapure water, with a resistivity of 18.2 MΩ·cm and total organic carbon (TOC) levels of <10 ppb, is recommended. Pure water - glass-distilled or deionized - may not be acceptable. *NOTE: UltraPure Water is available for purchase from Cayman (Item No. 400000).*
4. Materials used for **Buffer Preparation** and **Sample Preparation** (see pages 10 and 11, respectively)

INTRODUCTION

Background

Recombinant protein expression is a valuable tool in the production of large amounts of protein for both functional and structural studies. To facilitate purification and detection, recombinant proteins are often labeled with affinity tags, such as hexahistidine (6X-His), GST, and DYKDDDDK. Cell lysates and samples at different stages of purification are generally analyzed by SDS-PAGE to determine the expression of DYKDDDDK-tagged proteins, which can add several days to the purification and analysis protocol. A semi-quantitative screening assay would provide the ability to rapidly screen for DYKDDDDK-tagged proteins at each stage of expression and purification, permitting the user to determine if there is sufficient protein expression to continue with purification and to monitor loss or enrichment at each stage.

About This Assay

Cayman's DYKDDDDK-Tag Detection ELISA Kit is a competitive assay designed for the rapid, semi-quantitative screening of cell lysates, supernatants, and affinity column fractions for DYKDDDDK-tagged proteins (DYKDDDDK-proteins).

Principle of the Assay

This assay is based on the competition between free DYKDDDDK-protein and a DYKDDDDK-tagged protein linked to horseradish peroxidase (HRP) (a DYKDDDDK-HRP Tracer) for a limited number of DYKDDDDK-specific monoclonal antibody binding sites. The concentration of the DYKDDDDK-HRP Tracer is held constant while the concentration of free DYKDDDDK-protein (standard or sample) varies. Thus, the amount of tracer that is able to bind to the monoclonal antibody will be inversely proportional to the concentration of free DYKDDDDK-protein in the well. This monoclonal antibody-DYKDDDDK (either free or tracer) complex binds to the goat anti-mouse IgG that has been previously attached to the well. The plate is then washed to remove any unbound reagents. Addition of TMB Substrate Solution, followed by HRP Stop Solution produces a yellow colored product which can be measured spectrophotometrically. The intensity of this color, determined spectrophotometrically, is proportional to the amount of tracer bound to the well, which is inversely proportional to the amount of free DYKDDDDK-protein present in the well during the incubation; or

$$\text{Absorbance} \propto [\text{Bound DYKDDDDK Tracer}] \propto 1/[\text{DYKDDDDK-protein}]$$

A schematic description of the assay is shown in Figure 1, on page 8.

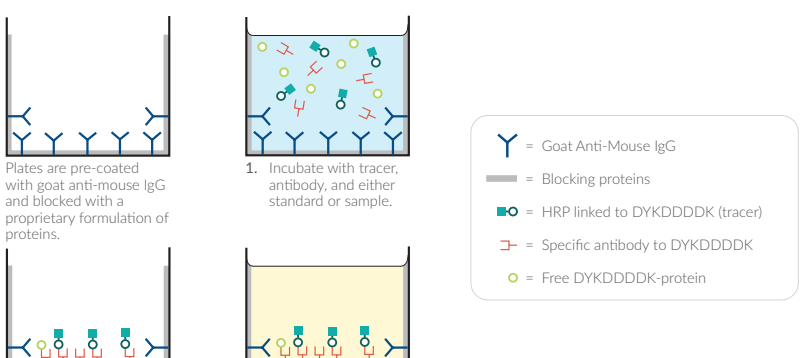


Figure 1. Schematic of the ELISA

Definition of Key Terms

Blk (Blank): background absorbance caused by the HRP reagent. The blank absorbance should be subtracted from the absorbance readings of all the other wells, including the non-specific binding (NSB) wells.

TA (Total Activity): total enzymatic activity of the DYKDDDDK HRP-linked tracer.

NSB (Non-Specific Binding): non-immunological binding of the tracer to the well. Even in the absence of specific antibody a very small amount of tracer still binds to the well; the NSB is a measure of this low binding.

B_0 (Maximum Binding): maximum amount of the tracer that the antibody can bind in the absence of free analyte.

%B/B_0 (%Bound/Maximum Bound): ratio of the absorbance of a sample or standard well to that of the maximum binding (B_0) wells.

Standard Curve: a plot of the %B/B_0 values *versus* concentration of a series of wells containing various known amounts of analyte.

Dynamic Range: the range in which the analyte is reliably quantifiable.

Lower Limit of Detection (LLOD): the smallest measure that can be detected with reasonable certainty for a given analytical procedure. The LLOD is defined as two standard deviations away from the mean zero value.

Dtn (Determination): one dtn is the amount of reagent used per well.

PRE-ASSAY PREPARATION

Buffer Preparation

Store all buffers at 4°C; they will be stable for approximately two months.

1. **Immunoassay Buffer C (1X) Preparation**

   Dilute the contents of each vial of Immunoassay Buffer C Concentrate (10X) (Item No. 401703) with 90 ml of pure water. Be certain to rinse the vial to remove any salts that may have precipitated. *NOTE: It is normal for the concentrated buffer to contain crystalline salts after thawing. These will completely dissolve upon dilution with water.*

2. **Wash Buffer Preparation**

   5 ml vial Wash Buffer (96-well kit; Item No. 400062): Dilute to a total volume of 2 liters with pure water and add 1 ml of Polysorbate 20 (Item No. 400035). Smaller volumes of Wash Buffer (1X) can be prepared by diluting the Wash Buffer Concentrate (400X) 1:400 and adding 0.5 ml of Polysorbate 20 per 1 L of Wash Buffer (1X).

   NOTE: Polysorbate 20 is a viscous liquid and cannot be measured by a regular pipette. A positive displacement pipette or a syringe should be used to deliver small quantities accurately.

Sample Preparation

Crude cell lysates contain materials that interfere in the assay and give false positives or erroneously high levels of DYKDDDDK-tagged protein. It is recommended that cell lysates be cleared by high speed centrifugation before assaying:

Reagents	
Compatible Buffers and Reagents	PBS (phosphate-buffered saline)
	PBS (1.0% NP-40)
	TBS (tris-buffered saline)
	M-PER protein extraction reagent
	Arginine-HCl elution reagent (<50 mM)
	Glycine, pH 3.0, elution reagent (<50 mM)
Incompatible Reagents	DYKDDDDK peptide (elution reagent)
	Arginine-HCl elution reagent (>50 mM)
	Glycine, pH 3.0, elution reagent (>50 mM)

Table 1. Buffers and reagents that have been tested and demonstrated to be compatible or incompatible with the assay.

Preparation of Assay-Specific Reagents

DYKDDDDK-Protein ELISA Standard

This kit contains one vial of DYKDDDDK-Protein ELISA Standard (Item No. 401564). As this is a rapid screening assay, and the immunoreactivity of different DYKDDDDK-tagged proteins is potentially variable, the standard is not designed to be used as a quantitative tool, but provide a means of monitoring protein gain or loss during the various purification steps.

Reconstitute the DYKDDDDK-Protein ELISA Standard with 1 ml Immunoassay Buffer C (1X). The concentration of this solution (the bulk standard) will be 50 µg/ml. Store this solution at 4°C; it will be stable for two weeks. For longer storage, we recommend the bulk standard be aliquoted and stored at -20°C or lower. Avoid repeated freeze/thaw cycles.

To prepare the standard for use in ELISA: Obtain eight clean test tubes and number them #1 through #8. Aliquot 350 µl Immunoassay Buffer C (1X) to the first tube and 250 µl Immunoassay Buffer C (1X) to all the other tubes. Transfer 150 µl of the bulk standard to tube #1 and mix thoroughly. Serially dilute the standard by removing 250 µl from tube #1 and placing in tube #2; mix thoroughly. Next, remove 250 µl from tube #2 and place it into tube #3; mix thoroughly. Repeat this process for tubes #4-8.

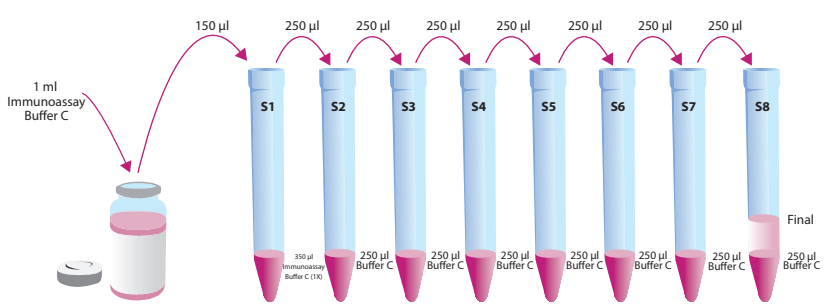


Figure 2. Preparation of the DYKDDDDK-protein standards

DYKDDDDK-HRP Tracer

Dilute the DYKDDDDK-HRP-Tracer (Item No. 401596) with 5 ml of Immunoassay Buffer C (1X). Store the diluted HRP Tracer at 4°C (**Do not freeze!**) and use within two weeks.

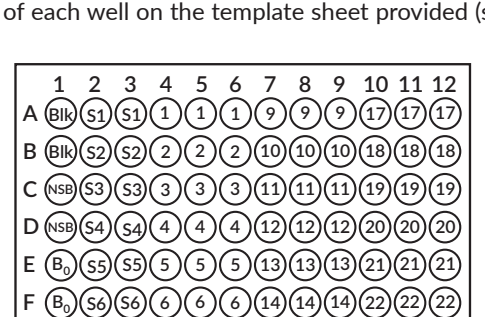
DYKDDDDK ELISA Monoclonal Antibody

Reconstitute the DYKDDDDK ELISA Monoclonal Antibody (Item No. 401562) with 6 ml Immunoassay Buffer C (1X). Store the reconstituted DYKDDDDK ELISA Monoclonal Antibody at 4°C. It should be stable for at least four weeks.

Plate Set Up

The 96-well plate(s) included with this kit is supplied ready to use. It is not necessary to rinse the plate(s) prior to adding the reagents. *NOTE: If you do not need to use all the strips at once, place the unused strips back in the plate packet and store at 4°C. Be sure the packet is sealed with the desiccant inside.*

There is no specific pattern for using the wells on the plate. A suggested plate format is shown in Figure 3, below. Each plate or set of strips should contain a minimum of two blanks (Blk), two non-specific binding wells (NSB), three maximum binding wells (B_0), and an eight point standard curve run in duplicate. *NOTE: Each assay must contain this minimum configuration in order to ensure accurate and reproducible results.* Each sample should be assayed at two dilutions and each dilution should be assayed in duplicate. For statistical purposes, we recommend assaying samples in triplicate. We suggest you record the contents of each well on the template sheet provided (see page 25).



Blk - Blank wells
TA - Total Activity wells
NSB - Non-Specific Binding wells
B_0 - Maximum Binding wells
S1-S8 - Standard 1-8 wells
1-24 - Sample wells

Figure 3. Sample plate format

Performing the Assay

Pipetting Hints

- Use different tips to pipette the standard, sample, and detection antibody.
- Before pipetting each reagent, equilibrate the pipette tip in that reagent (*i.e.*, slowly fill the tip and gently expel the contents, repeat several times).
- Do not expose the pipette tip to the reagent(s) already in the well.

Addition of the Reagents

1. **Immunoassay Buffer C**

   Add 100 µl Immunoassay Buffer C (1X) to NSB wells. Add 50 µl Immunoassay Buffer C (1X) to B_0 wells.

2. **DYKDDDDK-Protein ELISA Standard**

   Add 50 µl from tube #8 to both of the lowest standard wells (S8). Add 50 µl from tube #7 to each of the next two standard wells (S7). Continue with this procedure until all the standards are aliquoted. The same pipette tip should be used to aliquot all the standards. Before pipetting each standard, be sure to equilibrate the pipette tip in that standard.

3. **Samples**

   Add 50 µl of sample per well. Each sample should be assayed at a minimum of two dilutions. Each dilution should be assayed in duplicate (triplicate recommended).

4. **DYKDDDDK-HRP Tracer**

   Add 50 µl to each well except the Blk and TA wells.

5. **DYKDDDDK ELISA Monoclonal Antibody**

   Add 50 µl to each well except the NSB, Blk, and TA wells.

Incubation of the Plate

Cover each plate with plastic film (Item No. 400012) and incubate for 90 minutes at room temperature on an orbital shaker.

Development of the Plate

1. Empty the wells and rinse five times with Wash Buffer. Each well should be completely filled with Wash Buffer during each wash. Invert the plate between wash steps to empty the fluid from the wells. After the last wash, gently tap the inverted plate on absorbent paper to remove the residual Wash Buffer.
2. Add 150 µl of TMB Substrate Solution (Item No. 400074) to each well of the plate. Mix 10 µl of tracer with 90 µl of Immunoassay Buffer C (1X). Add 5 µl of this solution to TA well.
3. Cover the plate with plastic film and incubate for 30 minutes at room temperature in the dark on an orbital shaker.
4. DO NOT WASH THE PLATE. Add 50 µl of HRP Stop Solution (Item No. 10011355) to each well of the plate. Blue wells should turn yellow and colorless wells should remain colorless. *NOTE: The Stop Solution in this kit contains an acid. Wear appropriate protection and use caution when handling this solution.*

Reading the Plate

1. Wipe the bottom of the plate with a clean tissue to remove fingerprints, dirt, etc.
2. Read the plate at a wavelength of 450 nm.

ANALYSIS

Many plate readers come with data reduction software that plots data automatically. Alternatively a spreadsheet program can be used. The data should be plotted as either %B/B_0 versus log concentration using a four-parameter logistic fit or as logit B/B_0 versus log concentration using a linear fit. NOTE: Cayman Chemical has a computer spreadsheet available for data analysis. Please contact Technical Service or visit our website (www.caymanchem.com/analysis/elisa) to obtain a free copy of this convenient data analysis tool.

Calculations

Preparation of the Data

The following procedure is recommended for preparation of the data prior to graphical analysis.

NOTE: If the plate reader has not subtracted the absorbance readings of the blank wells from the absorbance readings of the rest of the plate, be sure to do that now.

1. Average the absorbance readings from the NSB wells.
2. Average the absorbance readings from the B_0 wells.
3. Subtract the NSB average from the B_0 average. This is the corrected B_0 or corrected maximum binding.
4. Calculate the B/B_0 (Sample or Standard Bound/Maximum Bound) for the remaining wells. To do this, subtract the average NSB absorbance from the S1 absorbance and divide by the corrected B_0 (from Step 3). Repeat for S2-S8 and all sample wells. (To obtain %B/B_0 for a logistic four-parameter fit, multiply these values by 100.)

NOTE: The TA values are not used in the standard curve calculations. Rather, they are used as a diagnostic tool. Low or no absorbance from a TA well could indicate a dysfunction in the enzyme-substrate system.

Plot the Standard Curve

Plot %B/B_0 for standards S1-S8 *versus* DYKDDDDK-protein concentration using linear (y) and log (x) axes and perform a 4-parameter logistic fit.

Alternative Plot - The data can also be linearized using a logit transformation. The equation for this conversion is shown below. *NOTE: Do not use %B/B_0 in this calculation.*

$$\text{logit } (B/B_0) = \ln [B/B_0/(1 - B/B_0)]$$

Plot the data as logit (B/B_0) *versus* log concentrations and perform a linear regression fit.

Determine the Sample Concentration

Calculate the B/B_0 (or %B/B_0) value for each sample. Determine the concentration of each sample using the equation obtained from the standard curve plot. *NOTE: Remember to account for any concentration or dilution of the sample prior to the addition to the well.* Samples with %B/B_0 values greater than 80% or less than 20% should be re-assayed as they generally fall out of the linear range of the standard curve. A 20% or greater disparity between the apparent concentration of two different dilutions of the same sample indicates interference which could be eliminated by purification.

To determine molar concentration of DYKDDDDK-tag in your samples, use the following formula:

$$[\text{DYKDDDDK}], \mu M = \frac{\text{concentration determined by ELISA } (\mu g/ml)}{9.58 \text{ (M.W. of the standard, kDa)}}$$

Performance Characteristics

This assay is semi-quantitative. Due to variability in immunodetection of the DYKDDDDK-tag on DYKDDDDK-tagged proteins, the sensitivity of this assay will vary depending on the protein being analyzed. The purified DYKDDDDK-tagged standard provided typically demonstrates a sensitivity of 0.5 µg/ml. If the tertiary structure of the experimental protein partially occludes the DYKDDDDK-tag, the sensitivity of the assay may be reduced.

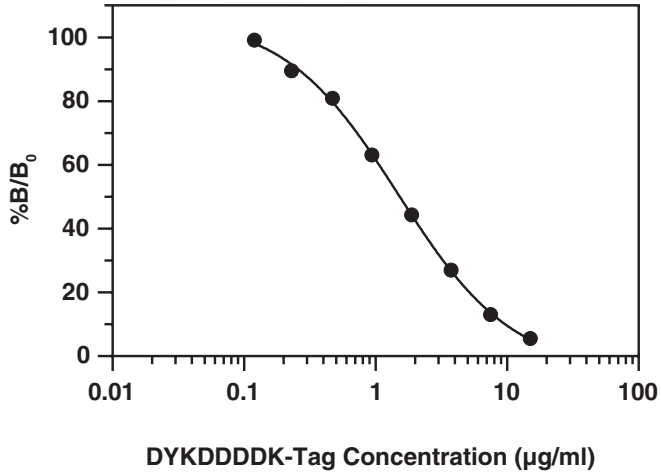
Representative Data

The standard curve presented here is an example of the data typically produced with this kit; however, your results will not be identical to these. You must run a new standard curve. Do not use the data below to determine the values of your samples.

Absorbance (450 nm) at 30 minutes

DYKDDDDK-Protein Standards (µg/ml)	Blank-Subtracted Absorbance	NSB Corrected Absorbance	%B/B_0	%CV* Intra-Assay Precision	*%CV* Inter-Assay Precision
NSB	0.005	--	--	--	--
B_0	0.777	0.772	--	--	--
15	0.048	0.043	5.6	8.4	1.9
7.5	0.105	0.100	13.0	4.1	2.3
3.75	0.213	0.208	27.0	5.5	1.9
1.88	0.347	0.342	44.3	6.4	2.1
0.94	0.492	0.487	63.1	6.5	2.3
0.47	0.629	0.624	80.9	10.2	4.7
0.23	0.696	0.691	89.5	12.9	7.5
0.12	0.770	0.765	99.1	38.3**	5.9
TA	0.615	--	--	--	--

Table 2. Typical results
*%CV represents the variation in concentration (not absorbance) as determined using a reference standard curve
**Evaluate data in this range with caution



Assay Range = 0.12-15 µg/ml (0.01-1.6 µM)
Sensitivity (defined as 80% B/B_0) = 0.5 µg/ml (0.05 µM)
Mid-point (defined as 50% B/B_0) = 1-2.5 µg/ml (0.1-0.26 µM)
Lower Limit of Detection = 0.05 µg/ml (0.005 µM)

The sensitivity and mid-point were derived from the standard curve shown above. The standard was diluted with Immunoassay Buffer C (1X).

Figure 4. Typical standard curve

Precision:

The intra-assay precision was determined by analyzing 24 replicates of three matrix controls (cell culture media) in a single assay.

Matrix Control (µg/ml)	%CV*
14.2	3.8
5.2	4.3
2	17.2

Table 3. Intra-assay variation

Matrix Control (µg/ml)	%CV*
13.5	11.4
5.3	12.4
1.98	15.4

Table 4. Inter-assay variation

*%CV represents the variation in concentration (not absorbance) as determined using a reference standard curve.

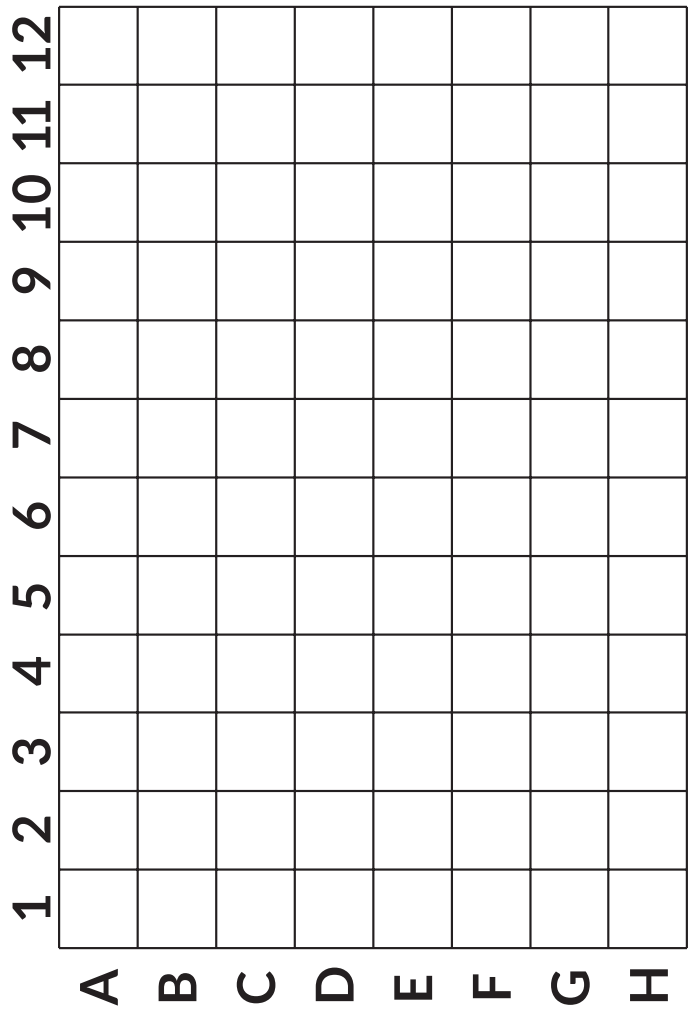
RESOURCES

Troubleshooting

Problem	Possible Causes
Erratic values; dispersion of duplicates	A. Trace organic contaminants in the water source B. Poor pipetting/technique
High NSB (10% of B_0)	A. Poor washing B. Exposure of NSB wells to specific antibody
Very low B_0	A. Trace organic contaminants in the water source B. Dilution error in preparing reagents
Low sensitivity (shift in dose response curve)	Standard is degraded
Analyses of two dilutions of a biological sample do not agree (*i.e.*, more than 20% difference)	Interfering substances are present

Procedure	Blk	TA	NSB	B_0	Standards/Samples
Reconstitute and Mix	Mix all reagents gently				
Immunoassay Buffer C (1X)	-	-	100 µl	50 µl	-
DYKDDDDK Standards/ Controls	-	-	-	-	50 µl
DYKDDDDK-HRP Tracer	-	-	50 µl	50 µl	50 µl
DYKDDDDK ELISA Monoclonal Antibody	-	-	-	50 µl	50 µl
Seal	Seal the plate and tap gently to mix				
Incubate	Incubate plate for 90 minutes at RT, shaking				
Aspirate	Aspirate wells and wash 5 x fill well volume (~300 µl) with Wash Buffer (1X)				
TMB Substrate	150 µl	150 µl	150 µl	150 µl	150 µl
DYKDDDDK-HRP Tracer	-	5 µl	--	--	--
Incubate	Incubate plate for 30 minutes at RT, shaking, sealed, protected from light				
HRP Stop Solution	50 µl	50 µl	50 µl	50 µl	50 µl
Read	Read O.D. at 450 nm				

Table 5. DYKDDDDK Assay Summary



Reference

1.. Maxey, K.M., Maddipati, K.R., and Birkmeier, J. Interference in enzyme immunoassays. *J. Clin. Immunoassay* **15**, 116-120 (1992).

NOTES

Warranty and Limitation of Remedy

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