Substance P ELISA Kit

Item No. 502630

www.caymanchem.com
Customer Service 800.364.9897
Technical Support 888.526.5351
1180 E. Ellsworth Rd · Ann Arbor, MI · USA

TABLE OF CONTENTS

GENERAL INFORMATION

Materials Supplied

Item Number	Item	96 wells Quantity/Size	Storage Temperature
401029	Substance P ELISA Antiserum	1 vial/100 dtn	-20°C
483750	Substance P AChE Tracer	1 vial/100 dtn	-20°C
483754	Substance P ELISA Standard	1 vial	-20°C
400060	ELISA Buffer Concentrate (10X)	2 vials/10 ml	RT
400062	Wash Buffer Concentrate (400X)	1 vial/5 ml	RT
400035	Polysorbate 20	1 vial/3 ml	RT
400004/400006	Mouse Anti-Rabbit IgG-Coated Plate	1 plate	4°C
400012	96-Well Cover Sheet	1 ea	RT
400050	Ellman's Reagent	3 vials/100 dtn	-20°C
400040	ELISA Tracer Dye	1 vial	RT
400042	ELISA Antiserum Dye	1 vial	RT

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.

The reagents in this kit have been tested and formulated to work exclusively with Cayman Chemical's Substance P ELISA Kit. This kit may not perform as described if any reagent or procedure is replaced or modified.

When compared to quantification by LC/MS or GC/MS, it is not uncommon for immunoassays to report higher analyte concentrations. While LC/MS or GC/MS analyses typically measure only a single compound, antibodies used in immunoassays sometimes recognize not only the target molecule, but also structurally related molecules, including biologically relevant metabolites. In many cases, measurement of both the parent molecule and metabolites is more representative of the overall biological response than is the measurement of a short-lived parent molecule. It is the responsibility of the researcher to understand the limits of both assay systems and to interpret their data accordingly.

If You Have Problems

Technical Service Contact Information

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

Email: 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 in the **Materials Supplied** section (see page 3) 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 between 405-420 nm
2. Adjustable pipettes and a repeating pipettor.
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 **Sample Preparation** (see page 12)

INTRODUCTION

Background

Substance P is a tachykinin neuropeptide and the primary ligand for neurokinin-1 (NK_1) receptors with roles in various physiological processes, including pain modulation, smooth muscle contraction, blood pressure regulation, water homeostasis, and renal function.[1-4] It is expressed in the central and peripheral nervous systems, including the enteric nervous system, and immune cells.[4] Substance P is released in the dorsal horn of the spinal cord in response to noxious stimuli at a magnitude proportional to the frequency and intensity of the stimulation.[5] Following noxious peripheral stimulation, it induces pain through NK_1 receptors located in the spinal cord. Substance P is also involved in depression, anxiety, seizures, and emesis among other functions.[4]

About This Assay

Cayman's Substance P ELISA Kit is a competitive assay that can be used for the quantification of Substance P in plasma, serum, urine and other sample matrices. The assay has a range of 9.8-1,250 pg/ml, an average sensitivity (80% B/B_0) of 34 pg/ml, and a lower limit of detection (LLOD) of 23 pg/ml.

Principle of the Assay

This assay is based on the competition between free Substance P and a Substance P-acetylcholinesterase (AChE) conjugate (Substance P AChE Tracer) for a limited number of Substance P polyclonal antiserum binding sites. Because the concentration of the Substance P AChE Tracer is held constant while the concentration of free Substance P varies, the amount of Substance P AChE Tracer that is able to bind to the polyclonal antiserum will be inversely proportional to the concentration of free Substance P in the well. This antibody-Substance P complex binds to the mouse anti-rabbit IgG that has been previously attached to the well. The plate is washed to remove any unbound reagents and Ellman's Reagent (which contains the substrate to AChE) is added to the well. The product of this enzymatic reaction has a distinct yellow color and absorbs strongly at 412 nm. The intensity of this color, determined spectrophotometrically, is proportional to the amount of Substance P AChE Tracer bound to the well, which is inversely proportional to the amount of free Substance P present in the well during the incubation, as described in the equation:

$$\text{Absorbance} \propto [\text{Bound Substance P AChE Tracer}] \propto 1/[\text{Substance P}]$$

A schematic of this process is shown in Figure 1, on page 8.

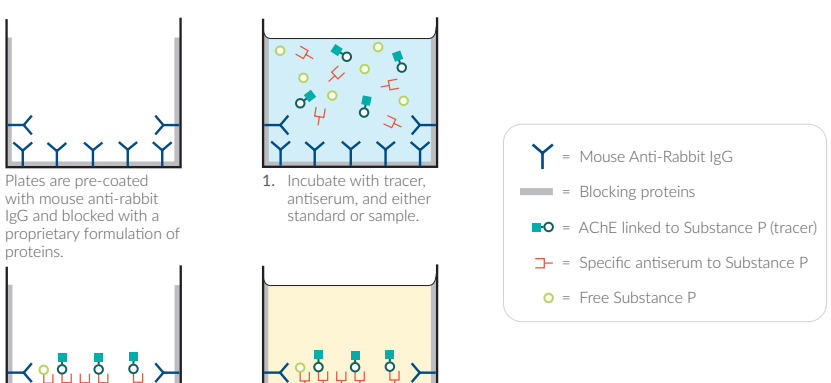


Figure 1. Schematic of the ELISA

Definition of Key Terms

Blk (Blank): background absorbance caused by Ellman's 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 AChE-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 the average absorbance 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.

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

Cross Reactivity: numerical representation of the relative reactivity of this assay towards structurally related molecules as compared to the primary analyte of interest. Biomolecules that possess similar epitopes to the analyte can compete with the assay tracer for binding to the primary antibody. Substances that are superior to the analyte in displacing the tracer result in a cross reactivity that is greater than 100%. Substances that are inferior to the primary analyte in displacing the tracer result in a cross reactivity that is less than 100%. Cross reactivity is calculated by comparing the mid-point (50% B/B_0) value of the tested molecule to the mid-point (50% B/B_0) value of the primary analyte when each is measured in assay buffer using the following formula:

$$\% \text{ Cross Reactivity} = \left[\frac{50\% \ B/B_0 \text{ value for the primary analyte}}{50\% \ B/B_0 \text{ value for the potential cross reactant}} \right] \times 100\%$$

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 a concentration two standard deviations higher than the mean zero value.

PRE-ASSAY PREPARATION

Store all diluted buffers at 4°C; they will be stable for at least two months. NOTE: It is normal for the concentrated buffer to contain crystalline salts after thawing. These will completely dissolve upon dilution with ultrapure water.

Buffer Preparation

1. **ELISA Buffer (1X) Preparation**

   Dilute the contents of one vial of ELISA Buffer Concentrate (10X) (Item No. 400060) with 90 ml of ultrapure water. Be certain to rinse the vial to remove any salts that may have precipitated.

2. **Wash Buffer (1X) Preparation**

   Dilute the contents of one vial of Wash Buffer Concentrate (400X) (Item No. 400062) with ultrapure water to a total volume of 2 L 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

In general, urine and tissue culture supernatant samples may be diluted with ELISA Buffer (1X) and added directly to the assay well. Dilute urine at least 1:2 prior to the assay. Plasma, serum, tissue homogenates, and other heterogeneous mixtures such as lavage fluids and aspirates often contain contaminants, which can interfere in the assay. It is best to check for interference before embarking on a large number of sample measurements.

To test for interference, dilute one or two test samples to obtain at least two different dilutions of each sample between within the linear portion of the standard curve. If the two different dilutions of the sample show good correlation (differ by 20% or less) in the final calculated Substance P concentration, purification is not required. If you do not see good correlation of the different dilutions, purification is advised. The **Purification Protocol**, on page 13, is one such method.

General Precautions

- All samples must be free of organic solvents prior to assay.
- Samples should be assayed immediately after collection; samples that cannot be assayed immediately should be stored at -80°C.
- Samples of rabbit origin may contain antibodies that interfere with the assay by binding to the mouse anti-rabbit plate. We recommend that all rabbit samples be purified prior to use in this assay.

SPE (C-18) Purification Protocol[6]

1. Aliquot a known amount of each sample into separate tubes. If samples need to be concentrated, a larger volume should be used.
2. Add 4 volumes of 4% acetic acid.
3. Activate a 6 ml SPE Cartridge (C-18) (Item No. 400020) by rinsing with 5 ml methanol and then with 5 ml ultrapure water. Do not allow the SPE cartridge (C-18) to dry.
4. Pass the sample slowly (about 2 ml/minute) through the SPE cartridge.
5. Wash the SPE cartridge with 10 ml of 4% acetic acid.
6. Prepare a solution of acetonitrile:1% trifluoroacetic acid (TFA) in water (60:40 v/v). Elute the Substance P by passing 3 ml of the acetonitrile:TFA solution through the SPE cartridge one milliliter at a time. Be certain to pause between each milliliter of solution as the reproducibility of the recovery is increased by the care taken during this step.
7. Dry the sample by vacuum centrifugation or under a gentle stream of nitrogen. It is imperative that all the organic solvent be removed as even trace quantities will adversely affect the ELISA.
8. Resuspend the samples in ELISA Buffer (1X) to their original volumes, and use for ELISA analysis. Samples can be concentrated in this step by using smaller volumes of buffer compared to the original sample volumes.

Spike and Recovery

Pooled heparin plasma, serum, and urine samples were spiked with Substance P. Plasma and serum were purified on SPE (C-18) cartridges following the protocol on page 13. All samples were diluted with ELISA Buffer (1X) and evaluated using the Substance P ELISA kit. The results are shown below. The error bars represent standard deviations obtained from multiple dilutions of each sample.

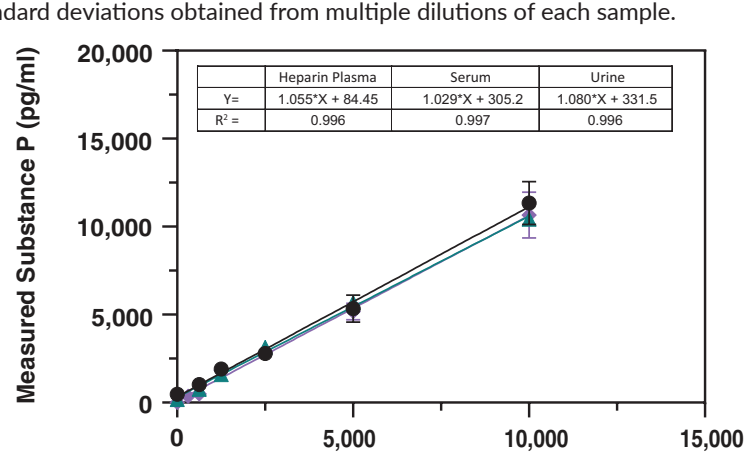


Figure 2. Spike and recovery of Substance P in various matrices

Linearity

Pooled heparin plasma, serum, and urine samples were spiked with 10 ng/ml Substance P. Plasma and serum were purified on SPE (C-18) cartridges following the protocol on page 13. All samples were diluted with ELISA Buffer (1X) and evaluated for linearity using the Substance P ELISA Kit.

Dilution	Measured Substance P (pg/ml)	Linearity (%)
Urine		
20	12,476	100
40	11,522	92
80	10,045	81
Serum		
40	10,809	100
80	10,271	95
160	10,188	94
Heparin Plasma		
40	11,573	100
80	9,741	84

Table 1. Linearity in various matrices

NOTE: Linearity has been calculated using the following formula:
*%Linearity = (Observed concentration value, dilution adjusted / First observed concentration value in the dilution series, dilution adjusted)*100*

ASSAY PROTOCOL

Preparation of Assay-Specific Reagents

Substance P ELISA Standard

Reconstitute the Substance P ELISA Standard (Item No. 483754) with 2 ml of ELISA Buffer (1X). The concentration of this solution (the bulk standard) will be 5 ng/ml. Store this solution at 4°C; it will be stable for approximately six weeks.

NOTE: If assaying culture media samples that have not been diluted with ELISA Buffer (1X), culture medium should be used in place of ELISA Buffer (1X) for dilution of the standard curve below.

To prepare the standard for use in ELISA: Obtain eight clean test tubes and number them #1 through #8. Aliquot 750 µl ELISA Buffer (1X) to tube #1 and 500 µl ELISA Buffer (1X) to tubes #2-8. Transfer 250 µl of the bulk standard (5 ng/ml) to tube #1 and mix thoroughly. Serially dilute the standard by removing 500 µl from tube #1 and placing in tube #2; mix thoroughly. Next, remove 500 µl from tube #2 and place it into tube #3; mix thoroughly. Repeat this process for tubes #4-8. These diluted standards should not be stored for more than 24 hours.

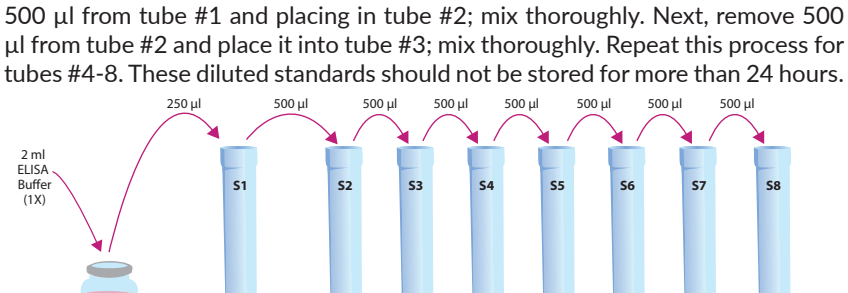


Figure 3. Preparation of the Substance P standards

Substance P AChE Tracer

Reconstitute the Substance P AChE Tracer (Item No. 483750) with 6 ml of ELISA Buffer (1X). Store the reconstituted Substance P AChE Tracer at 4°C (*do not freeze!*). It will be stable for at least four weeks. A 20% surplus of tracer has been included to account for any incidental losses.

Tracer Dye Instructions (optional)

This dye may be added to the tracer, if desired, to aid in visualization of tracer-containing wells. Add the dye to the reconstituted tracer at a final dilution of 1:100 (add 60 µl of dye to 6 ml tracer). *Do not store tracer with dye.*

Substance P ELISA Antiserum

Reconstitute the Substance P ELISA Antiserum (Item No. 401029) with 6 ml of ELISA Buffer (1X). Store the reconstituted Substance P ELISA Antiserum at 4°C (*do not freeze!*). It will be stable for at least four weeks. A 20% surplus of antibody has been included to account for any incidental losses.

Antiserum Dye Instructions (optional)

This dye may be added to the antiserum, if desired, to aid in visualization of antiserum-containing wells. Add the dye to the reconstituted antiserum at a final dilution of 1:100 (add 60 µl of dye to 6 ml antiserum). *Do not store antiserum with dye.*

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.*

Each plate or set of strips must contain a minimum of two Blk, two NSB, and three B_0 wells, 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 a minimum of two dilutions, and each dilution should be assayed at least in duplicate.

A suggested plate format is shown in Figure 4, below. The user may vary the location and type of wells present as necessary for each particular experiment. The plate format provided below has been designed to allow for easy data analysis using a convenient spreadsheet offered by Cayman (see page 22 for more details). We suggest you record the contents of each well on the template sheet provided (see page 29).

	1	2	3	4	5	6	7	8	9	10	11	12
A	Blk	S1	S1	1	1	1	9	9	9	17	17	17
B	Blk	S2	S2	2	2	2	10	10	10	18	18	18
C	NSB	S3	S3	3	3	3	11	11	11	19	19	19
D	NSB	S4	S4	4	4	4	12	12	12	20	20	20
E	B_0	S5	S5	5	5	5	13	13	13	21	21	21
F	B_0	S6	S6	6	6	6	14	14	14	22	22	22
G	B_0	S7	S7	7	7	7	15	15	15	23	23	23
H	TA	S8	S8	8	8	8	16	16	16	24	24	24

Blk - Blank
TA - Total Activity
NSB - Non-Specific Binding
B_0 - Maximum Binding
S1-S8 - Standards 1-8
1-24 - Samples

Figure 4. Sample plate format

Performing the Assay

Pipetting Hints

- Use different tips to pipette each reagent.
- 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. **ELISA Buffer (1X)**

   Add 100 µl ELISA Buffer (1X) to NSB wells. Add 50 µl ELISA Buffer (1X) to B_0 wells. If culture medium was used to dilute the standard curve, add 50 µl culture medium to NSB and B_0 wells and 50 µl ELISA Buffer (1X) to NSB wells.

2. **Substance P 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. Before pipetting each standard, be sure to equilibrate the pipette tip in that standard.

3. **Samples**

   Add 50 µl of sample per well.

4. **Substance P AChE Tracer**

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

5. **Substance P ELISA Antiserum**

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

Well	ELISA Buffer (1X)	Standard/ Sample	Tracer	Antiserum
Blk	-	-	-	-
TA	-	-	5 µl (at devl. step)	-
NSB	100 µl	-	50 µl	-
B_0	50 µl	-	50 µl	50 µl
Std/Sample	-	50 µl	50 µl	50 µl

Table 2. Pipetting summary

Incubation of the Plate

Cover each plate with a 96-Well Cover Sheet (Item No. 400012) and incubate for 18-22 hours at 4°C.

Development of the Plate

1. Reconstitute Ellman's Reagent (Item No. 400050) immediately before use with 20 ml of ultrapure water. *NOTE: Reconstituted Ellman's Reagent is unstable and should be used the same day it is prepared; protect the Ellman's Reagent from light when not in use. Extra vials of the reagent have been provided should a plate need to be re-developed or multiple assays run on different days.*
2. Empty the wells and rinse five times with ~300 µl Wash Buffer (1X).
3. Add 200 µl of Ellman's Reagent to each well.
4. Add 5 µl of the reconstituted tracer to the TA wells.
5. Cover the plate with the 96-Well Cover Sheet. Optimum development is obtained by using an orbital shaker equipped with a large, flat cover to allow the plate(s) to develop in the dark. This assay typically develops (*i.e.*, B_0 wells ≥0.3 A.U. (Blk subtracted)) in 90-120 minutes.

Reading the Plate

1. Wipe the bottom of the plate with a clean tissue to remove fingerprints, dirt, *etc.*
2. Remove the plate cover being careful to keep Ellman's Reagent from splashing on the cover. *NOTE: Any loss of Ellman's Reagent will affect the absorbance readings.*
3. Read the plate at a wavelength between 405 and 420 nm. The absorbance may be checked periodically until the B_0 wells have reached a minimum of 0.3 A.U. (Blk subtracted). The plate should be read when the absorbance of the B_0 wells in the range of 0.3-2.0 A.U. (Blk subtracted). If the absorbance of the wells exceeds 2.0, wash the plate, add fresh Ellman's Reagent, and let it develop again.

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 Blk 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* Substance P concentration using linear (y) and log (x) axes and perform a four-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 dilution of the sample concentration prior to its 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.

NOTE: If there is an error in the B_0 wells, plot the absorbance values instead of %B/B_0 to calculate sample concentrations.

Performance Characteristics

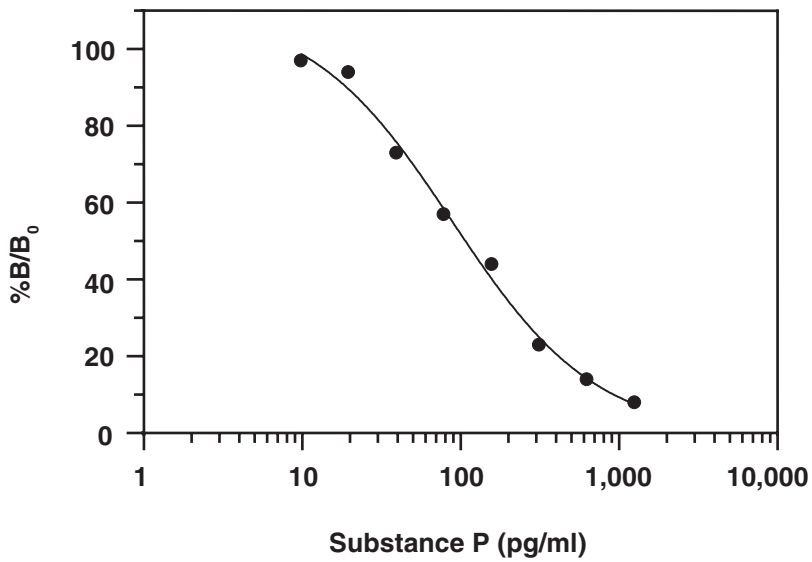
The standard curve presented here is an example of the data typically produced with this kit; however, your results will not be identical to these. You must run a new standard curve. Do not use the data below to determine the values of your samples. Your results could differ substantially.

Substance P Standard	Blk-subtracted Absorbance	NSB-corrected Absorbance	%B/B_0	%CV Intra-Assay*	%CV Inter-Assay*
NSB	0.006	--	--	--	--
B_0	0.952	0.946	--	--	--
TA	0.876	--	--	--	--
1,250.0	0.085	0.079	8.4	14.6	24.4**
625.0	0.142	0.136	14.4	11.5	5.8
312.5	0.228	0.222	23.5	5.3	6.8
156.3	0.425	0.419	44.3	8.2	6.2
78.1	0.546	0.540	57.1	11.8	9.1
39.1	0.697	0.691	73.0	13.0	13.7
19.5	0.901	0.895	94.6	31.5**	29.3**
9.8	0.934	0.928	98.1	29.6**	37.7**

Table 3. 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 = 9.8-1,250 pg/ml
Sensitivity (defined as 80% B/B_0) = 32.9 pg/ml
Mid-point (defined as 50% B/B_0) = 108 pg/ml
Lower Limit of Detection (LLOD) = 23.2 pg/ml

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

Figure 5. Typical standard curve

Cross Reactivity:

Compound	Cross Reactivity
Substance P	100%
Hemokinin-1	100%
Eledoisin	<0.01%
NKA (substance K)	<0.01%
NKB (neuromedin K)	0.01%

Table 4. Cross reactivity of the Substance P ELISA

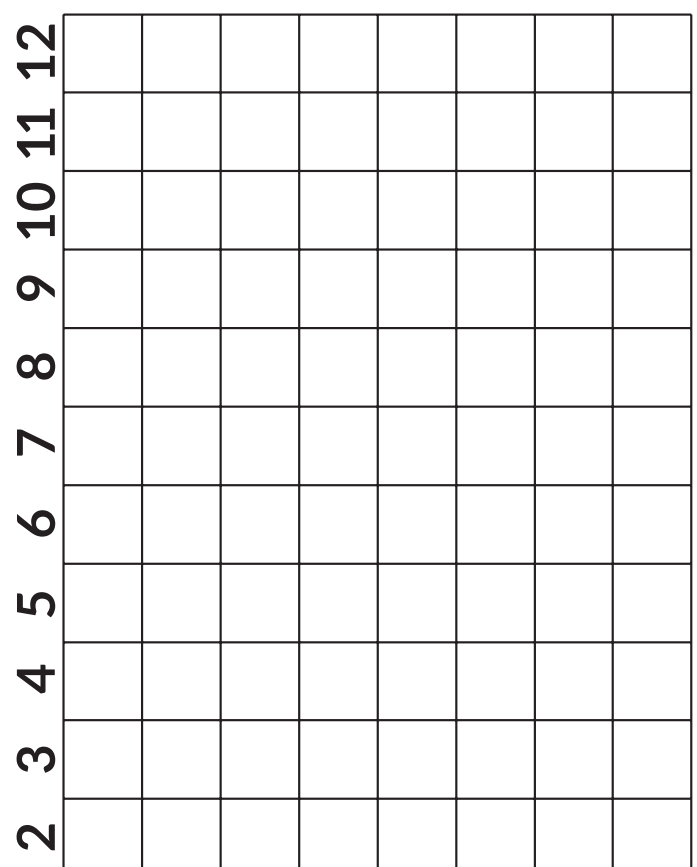
RESOURCES

Troubleshooting

Problem	Possible Causes
Erratic values; dispersion of replicates	A. Trace organic contaminants in the water 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. Plate requires additional development time C. 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; purification is needed
Only TA wells develop	A. Trace organic contaminants in the water B. Tracer or antiserum were not added to the wells

References

1. Jessell, T.M. The role of Substance P in sensory transmission and pain perception, *in* Neurosecretion and Brain Peptides. Raven Press, New York, 189-198 (1981).
2. Pernow, B. Substance P. *Pharmacol. Rev.* **35(2)**, 85-141 (1983).
3. Gether, U., Johansen, T.E., Snider, R.M., *et al.* Different binding epitopes on the NK1 receptor for substance P and non-peptide antagonist. *Nature.* **362(6418)**, 345-348 (1993).
4. Muñoz, M. and Coveñas, R. Involvement of substance P and the NK-1 receptor in human pathology. *Amino Acids* **46(7)**, 1727-1750 (2014).
5. Mantyh, P.H. Neurobiology of substance P and the NK1 receptor. *J. Clin. Psychiatry* **63(Suppl 11)**, 6-10 (2002).
6. Fehder, W.P., Ho, W.-Z., Campbell, D.E., *et al.* Development and evaluation of a chromatographic procedure for partial purification of substance P with quantification by an enzyme immunoassay. *Clinical and Diagnostic Laboratory Immunology* **5**, 303-307 (1998).



NOTES

Warranty and Limitation of Remedy

Buyer agrees to purchase the material subject to Cayman's Terms and Conditions. Complete Terms and Conditions including Warranty and Limitation of Liability information can be found on our website.