VALIDATION DATA

Substance P ELISA Kit (Express)

Item No. 502892

The Substance P ELISA Kit (Express) was assessed using a variety of tests and procedures to validate its performance for the quantitative measurement of Substance P in biological matrices commonly used with this kit. The validation experiments were designed to evaluate assay sensitivity, working range, precision, accuracy, and matrix compatibility under the conditions described in the kit booklet.

The data presented in this document describe the performance characteristics of the assay and are intended to assist users in experimental planning and interpretation of results.

Parameter	Performance Metric
Detection method	Colorimetric (450 nm)
Assay duration	2.5 hours
Lower Limit of Detection (LLOD)	5.7 pg/ml
Sample Volume	100 µl
Species compatibility	Multiple species*
Validated species	Human, horse, pig
Validated matrices	Plasma (EDTA and heparin) and serum

**NOTE: This assay is expected to be compatible with samples from a variety of species; however, performance has not been validated across all species.*

Assay Range and Sensitivity

The assay range is 15.6-2,000 pg/ml when determined under the conditions described in the kit protocol.

The lower limit of detection (LLOD) represents the lowest concentration that can be reliably distinguished from the background signal. The LLOD for the assay is 5.7 pg/ml.

The sensitivity of this assay is determined as a value corresponding to 80% B/B_0 of the standard curve. The sensitivity of the assay is 33 pg/ml.

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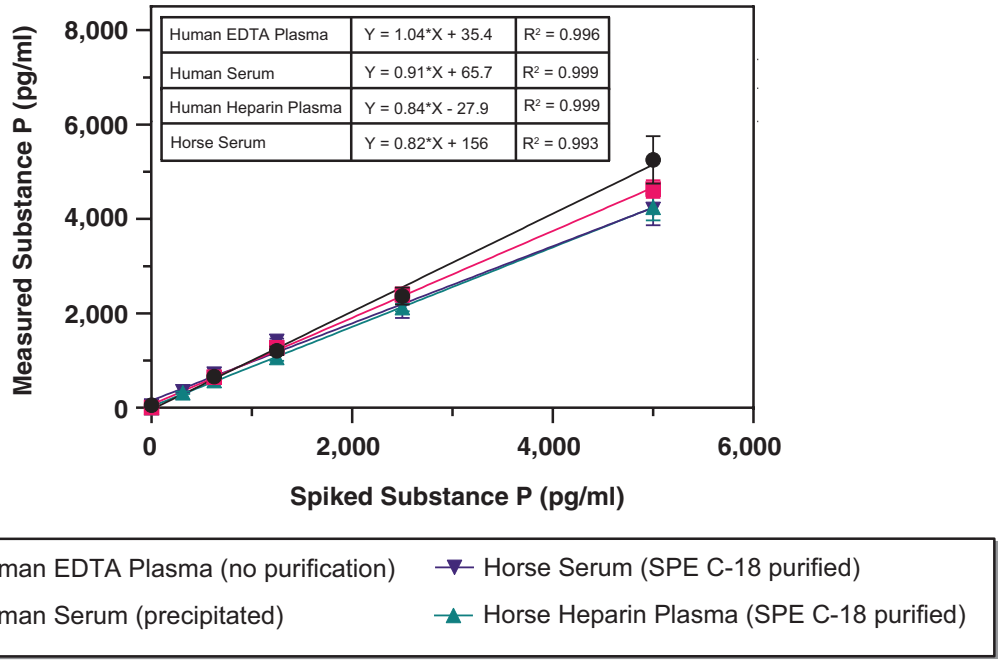
Spike and Recovery

Spike and recovery experiments are conducted to assess assay accuracy in the presence of complex biological matrix components. In these experiments, known quantities of analyte are spiked into the sample. The samples are then processed according to the kit booklet, diluted with assay buffer, and analyzed using the assay. Measured concentrations are plotted as a function of spiked analyte concentration. The slope of the line multiplied by 100 indicates an average percent recovery. A recovered concentration within 80-120% of the expected concentration is acceptable. Values beyond this range could indicate the presence of assay interferants.



Spike and recovery of Substance P in various matrices. Human serum, heparin and EDTA plasma, and horse serum were spiked with Substance P, processed as described in the Sample Preparation section of the kit booklet, serially diluted with assay buffer, and evaluated using the Substance P ELISA Kit (Express). The error bars indicate standard deviations from multiple dilutions of the same sample.

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Linearity

Linearity experiments are performed with biological samples spiked with high amounts of analyte to confirm that multiple dilutions of the sample yield similar measured concentrations of the analyte after dilution adjustment.

Dilution Factor	Measured Substance P, Dilution Adjusted (pg/ml)	Linearity%
Human heparin plasma spiked with 25 ng/ml		
100	24,291	100
200	25,571	105
400	24,846	102
Human EDTA plasma spiked with 10 ng/ml		
40	9,666	100
80	9,776	101
160	9,857	102
Human serum spiked with 10 ng/ml		
25	10,029	100
50	9,555	95.3
100	9,205	91.8
200	8,875	88.5
Horse serum spike with 5 ng/ml (SPE C-18-purified)		
16	4,638	100
32	4,149	89.5
64	3,820	82.4
128	3,891	83.9

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

Linearity in various samples. Human EDTA plasma, heparin plasma, and serum, and horse serum were spiked with Substance P, processed as described in the Sample Preparation section of the kit booklet, serially diluted with assay buffer, and evaluated for linearity using the Substance P ELISA Kit (Express).

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Precision

The coefficient of variation (CV) is a measurement of consistency between sample replicates. Precision was evaluated both within a single assay run (intra-assay) and across multiple runs on different days (inter-assay), using controls prepared from human serum and EDTA plasma.

Control	Measured Substance P (pg/ml)	%CV
Control 1 (spiked plasma)	9,672	3.0
Control 2 (spiked plasma)	4,134	3.2
Control 3 (spiked serum)	463	10.9

Intra-Assay Precision: To assess intra-assay precision, a series of 24 (Control 1 and 2) and 15 (Control 3) replicate measurements were performed on the same day. The measured values and CVs are listed in the table above.

Control	Measured Substance P (pg/ml)	%CV
Control 1 (spiked plasma)	8,040	16.9
Control 2 (spiked plasma)	3,775	9.9
Control 3 (spiked serum)	350	5.1

Inter-Assay Precision: To assess inter-assay precision, a series of eight replicate measurements were performed on different days under the same experimental conditions. The measured values and CVs are listed in the table above.

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