VALIDATION DATA

DHEA-S ELISA Kit

Item No. 502600

The DHEA-S ELISA Kit was validated to assess its performance for the quantitative measurement of DHEA-S in plasma, serum, urine, saliva, and cell culture supernatants, 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 describes the performance characteristics of the assay and is intended to assist users in experimental planning and interpretation of results.

Parameter	Performance Metric
Detection method	Colorimetric (414 nm)
Assay duration	3 hours
Sample volume	50 µl
Compatibility	Multi-species
Validated species	Human, monkey, horse, ovine, bovine, chicken, cat, dog, guinea pig
Validated matrices	Plasma, serum, urine, saliva, and cell culture supernatants

Assay Range and Sensitivity

The assay range is 31.3-4,000 pg/ml, under the conditions described in the kit protocol.

The lower limit of detection (LLOD) represents the lowest concentration of DHEA-S that can be reliably distinguished from the background signal. The LLOD for the assay is 30 pg/ml.

The sensitivity of this assay is determined as a value corresponding to 80% B/B_0 of the standard curve. The average sensitivity of this assay is 96 pg/ml.

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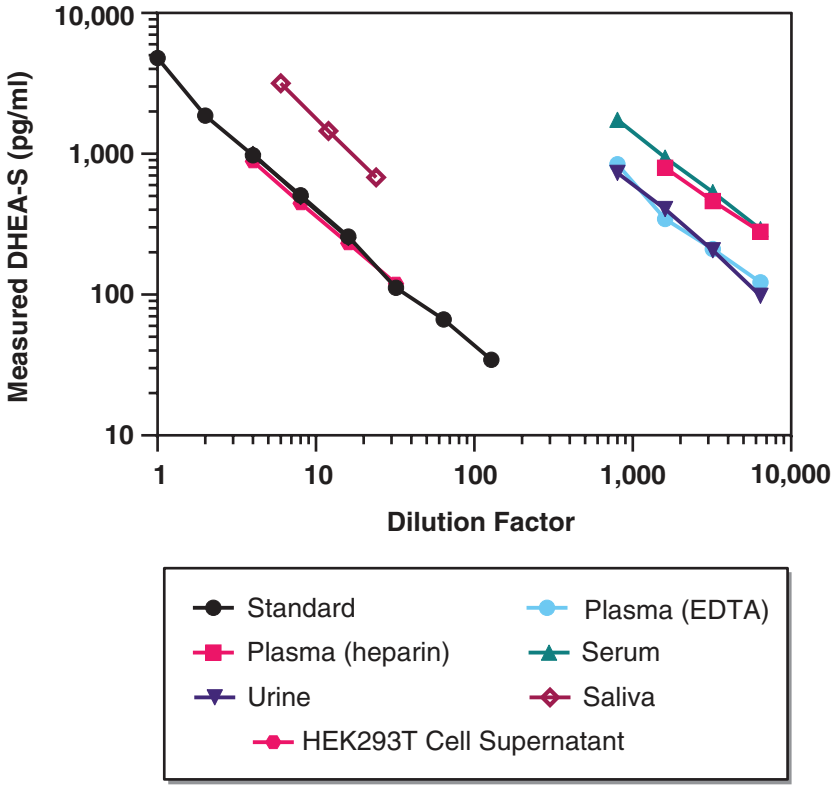
Parallelism

Parallelism experiments are performed to evaluate the relationship between the dose-response curve of various samples and the calibration standard series, to show that the native analyte in the sample matrix is immunologically similar to the reference standard. Parallelism experiments also assess the effect of sample matrices on analyte measurement and confirm that dilution of biological sample yields proportional changes in measured concentration.



Parallelism of human and cell culture samples. Human plasma, serum, urine, and saliva samples, and HEK293T cell supernatant were diluted with Assay Buffer and analyzed using the DHEA-S ELISA Kit. Measured concentrations were plotted as a function of sample dilution.

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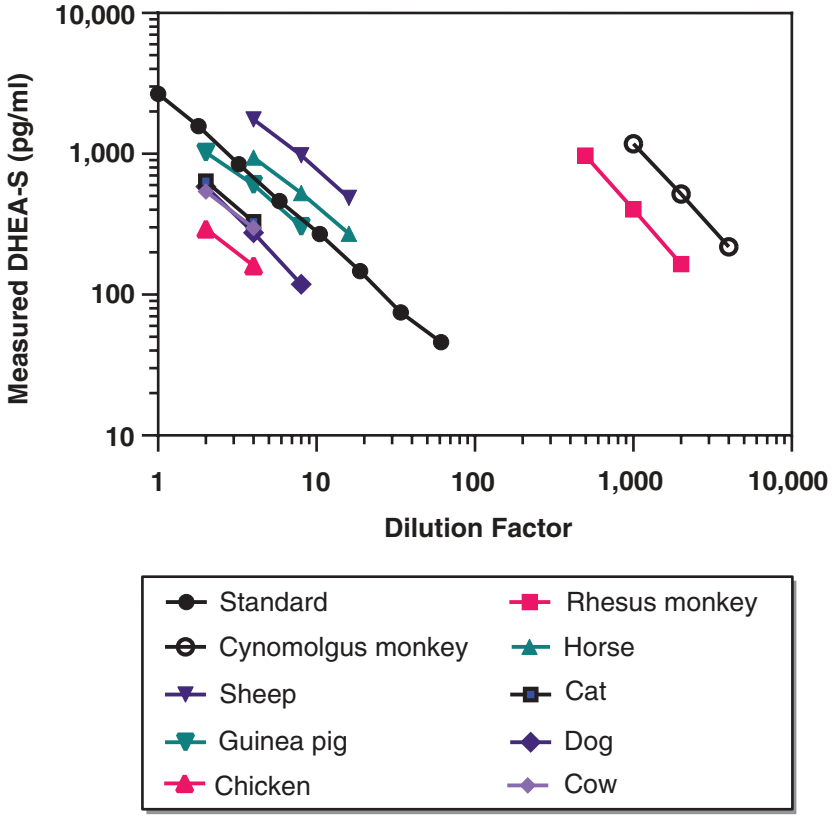

Parallelism of animal sera. Animal serum samples were diluted with Assay Buffer and analyzed using the DHEA-S ELISA Kit. Measured concentrations were plotted as a function of sample dilution.

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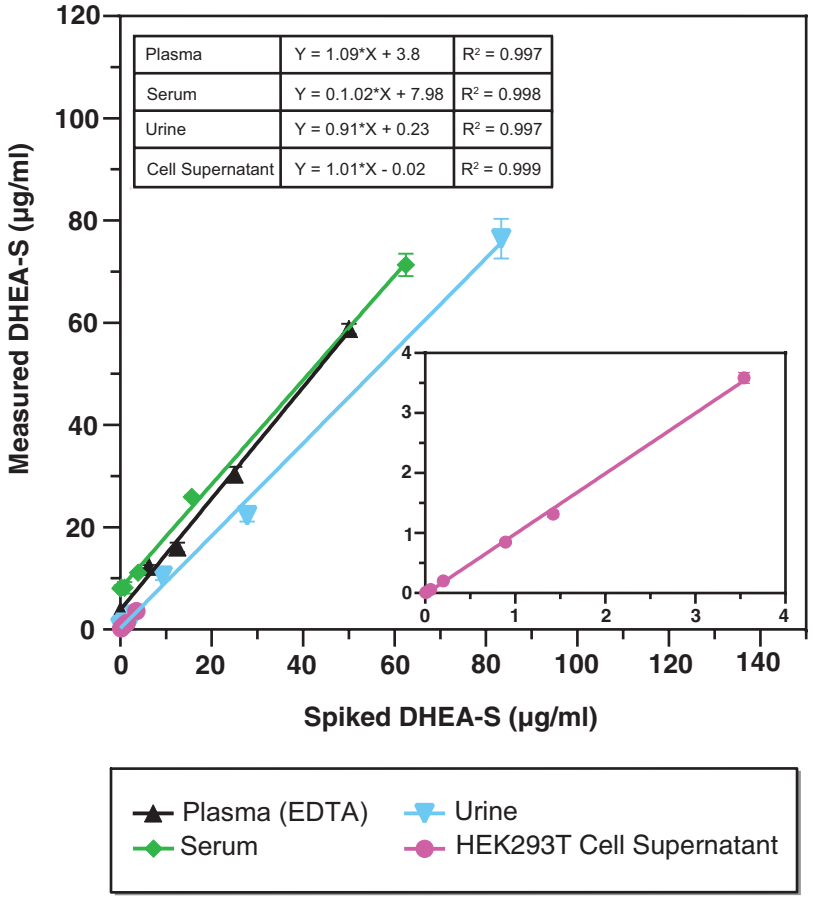
Spike and Recovery

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 as recommended in 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 recovery within 80-120% of the expected concentration (*i.e.*, the spiked concentration) is acceptable. Values beyond this range could indicate the presence of assay interferants.



Spike and recovery of DHEA-S in human samples and HEK293T cell supernatant. Pooled human plasma (EDTA), serum, and urine and HEK293T cell supernatant were spiked with known amounts of DHEA-S, diluted with Assay Buffer, and evaluated using the DHEA-S ELISA Kit. Measured concentrations were plotted as a function of spiked concentrations. The error bars represent standard deviations obtained from multiple dilutions of the same sample.

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PRODUCT INFORMATION

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.

Human plasma, urine, and saliva were spiked with DHEA-S, serially diluted with Assay Buffer, and evaluated for linearity using the DHEA-S ELISA Kit.

Dilution Factor	Measured Concentration (µg/ml)	% Linearity
Human plasma (EDTA) spiked with 25 µg/ml DHEA-S		
30,000	28.7	100
60,000	31.6	110
120,000	30.7	107
Human urine spiked with 83 µg/ml DHEA-S		
150,000	74.4	100
300,000	74.0	99.4
600,000	80.9	109
Human saliva spiked with 50 µg/ml DHEA-S		
80,000	48.9	100
160,000	47.8	97.8
320,000	44.6	91.2

Linearity in plasma, urine, and saliva samples

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) x 100.

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PRODUCT INFORMATION

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 (inter-assay) on different days, using controls prepared from pooled human plasma and urine samples.

Intra-Assay Precision: To assess intra-assay precision, a series of 24 replicate measurements were performed on the same day. The measured values and the coefficients of variation (CVs) are listed in the table below.

Controls	Measured DHEA-S (ng/ml)	%CV
Control 1 (urine)	16,180	11.5
Control 2 (plasma)	1,016	9.4

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

Controls	Measured DHEA-S (ng/ml)	%CV
Control 1 (urine)	11,061	13.3
Control 2 (plasma)	899	10.6

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