VALIDATION DATA

Cortisol ELISA Kit (Express)

Item No. 500370

The Cortisol ELISA Kit (Express) was assessed using a variety of tests and procedures to validate its performance for the quantitative measurement of cortisol in plasma, serum, urine, feces, hair, tissue, and cell culture supernatant, 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.

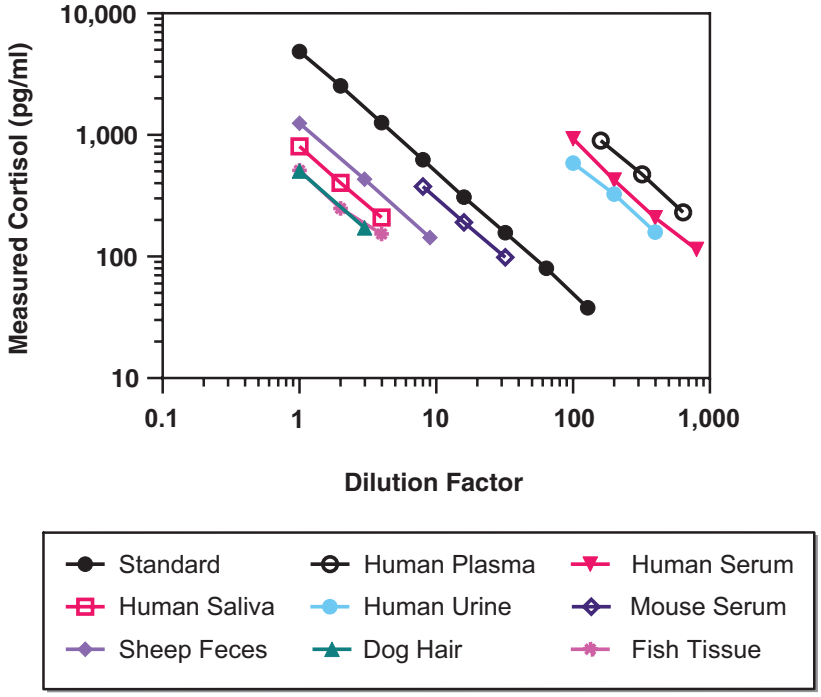
The data presented in this document describe the performance characteristics of the assay and are intended to assist users in experimental planning and interpretation of results.

Assay Range and Sensitivity

The assay range is 39.1-5,000 pg/ml. The lower limit of detection (LLOD) represents the lowest concentration of cortisol that can be reliably distinguished from the background signal. The LLOD for the assay is 20 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 kit is 80 pg/ml.

Parallelism

Parallelism experiments are performed to evaluate a relationship between the dose-response curve of various samples and a 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 effects of sample matrices on analyte measurement and confirm that dilution of biological samples yields proportional changes in measured concentration.



Parallelism of various matrices. Samples were processed as described in the Sample Preparation section of the kit booklet, serially diluted with ELISA Buffer (1X), and analyzed using the Cortisol ELISA Kit (Express). Measured concentrations were plotted as a function of sample dilution.

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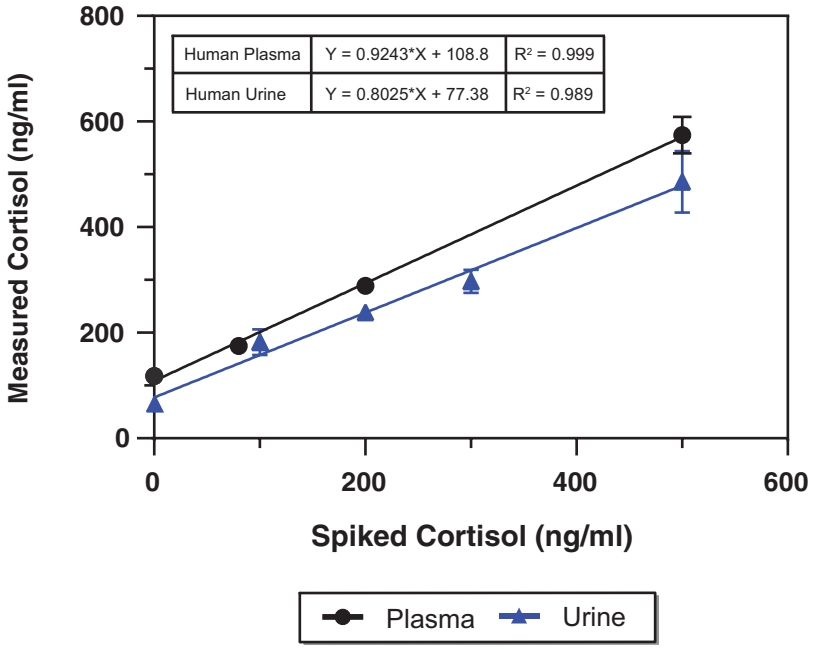
Spike and Recovery

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, 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 plus base concentration) is acceptable. Values beyond this range could indicate the presence of assay interferants.



Spike and recovery of cortisol in human plasma and urine. Human plasma and urine were spiked with cortisol, processed as described in the Sample Preparation section of the kit booklet, serially diluted with ELISA Buffer (1X), and evaluated using the Cortisol ELISA Kit (Express). The error bars represent standard deviations obtained from multiple dilutions of the same sample.

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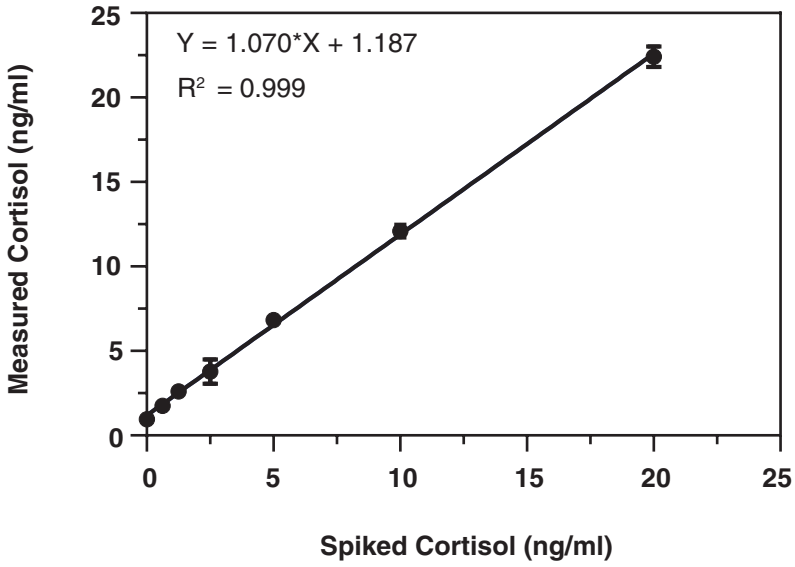

Spike and recovery of cortisol in human saliva. Human saliva was spiked with known amounts of cortisol, processed as described in the Sample Preparation section of the kit booklet, serially diluted with ELISA Buffer (1X), and evaluated using the Cortisol ELISA Kit (Express).

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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.

Human plasma, urine, and saliva, sheep feces, and fish tissue were spiked with cortisol, processed as described in the Sample Preparation section, serially diluted with ELISA Buffer (1X), and evaluated for linearity using the Cortisol ELISA Kit.

Dilution Factor	Measured Concentration (ng/ml)	Linearity (%)
Human plasma spiked with 500 ng/ml cortisol		
800	551	100
1,600	614	111
3,200	557	101
Human urine spiked with 300 ng/ml cortisol		
400	330	100
800	294	89
1,600	286	87
Human saliva spiked with 20 ng/ml cortisol		
20	23.3	100
40	22.4	96
80	22.0	94
Sheep feces spiked with 800 pg/mg of cortisol		
80	604	100
160	592	98
320	596	99
Fish tissue spiked with 100 ng/ml cortisol		
100	101	100
200	99.4	98.4
400	95.5	94.6

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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