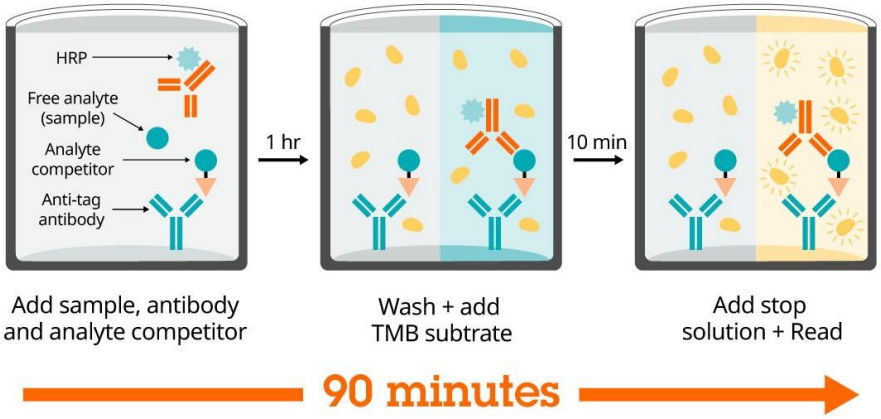
ab324547 – Human Cortisol SimpleStep ELISA® Kit – Extracellular (Hydrocortisone)

For the quantitative measurement of Cortisol in human serum, plasma (citrate), plasma (EDTA), plasma (heparin), urine, milk, saliva, and cell culture supernatant.
For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit: www.abcam.com/ab324547



Storage and Stability: Store kit at 2-8°C immediately upon receipt. Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in the Standard Preparation and Reagent preparation sections.

Materials Supplied

Item	Quantity 1 x 96 tests	Quantity 10 x 96 tests	Storage Condition
Human Cortisol Lyophilized CaptSure™ Conjugate	1 Vial	10 x 1 Vial	+4°C
Human Cortisol HRP Conjugate 50X	80 µL	10 x 80 µL	+4°C
Human Cortisol Lyophilized Standard	2 Vials	10 x 2 Vials	+4°C
Antibody Diluent 4BI	8 mL	10 x 8 mL	+4°C
Cell Extraction Buffer SSW	10 mL	2 X 50 mL	+4°C
Sample Diluent NS*	12 mL	-	+4°C
Wash Buffer PT 10X	20 mL	200 mL	+4°C
TMB Development Solution	12 mL	120 mL	+4°C
Stop Solution	12 mL	120 mL	+4°C
SimpleStep Pre-Coated 96-Well Microplate	96 wells	10 x 96 wells	+4°C
Plate Seal	1	10	+4°C

*Sample Diluent NS is provided but not necessary for this product.

Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully utilize this assay:

- Microplate reader capable of measuring absorbance at 450 or 600 nm.
- Deionized water.
- Multi- and single-channel pipettes.
- Tubes for standard dilution.
- Plate shaker for all incubation steps.
- Method for determining total protein concentration in sample (BCA assay recommended).
- Optional: Phenylmethylsulfonyl Fluoride (PMSF) (or other protease inhibitors).

Reagent Preparation

Equilibrate all reagents to room temperature (18-25°C) prior to use. The kit contains enough reagents for 96 wells. The sample volumes below are sufficient for 48 wells (6 x 8-well strips); adjust volumes as needed for the number of strips in your experiment.

Prepare only as much reagent as is needed on the day of the experiment. The CaptSure and HRP Conjugates have only been tested for stability in the provided lyophilized and 50X formulation.

1X Cell Extraction Buffer SSW: Prepare 1X Cell Extraction Buffer SSW by diluting Cell Extraction Buffer SSW Stock to 1X with 1X Wash Buffer PT. To make 10 mL 1X Cell Extraction Buffer SSW combine 9.6 mL 1X Wash Buffer PT and 0.4 mL Cell Extraction Buffer SSW Stock. Mix thoroughly and gently. If required protease inhibitors can be added.

1X Wash Buffer PT: Prepare 1X Wash Buffer PT by diluting Wash Buffer PT 10X with deionized water. To make 50 mL 1X Wash Buffer PT combine 5 mL Wash Buffer PT 10X with 45 mL deionized water. Mix thoroughly and gently.

CaptSure Conjugate: To reconstitute the lyophilized CaptSure conjugate, centrifuge at 10,000 g for 2 minutes. Add 100 µL of Cell Extraction Buffer SSW, let sit at room temperature for 10 minutes and resuspend well by inverting the tube by hand and gently pipetting. Unused reconstituted conjugate can be stored frozen at -20°C. Avoid repeated freeze-thaw cycles.

CaptSure Conjugate Solution: Prepare CaptSure Conjugate Solution by diluting the reconstituted CaptSure Conjugate in Antibody Diluent 4BI. To make 2 mL of the Capture Conjugate Solution combine 40 µL 50X CaptSure Conjugate with 1.96 mL Antibody Diluent 4BI. Mix thoroughly and gently.

HRP Conjugate Solution: Prepare HRP Conjugate Solution by diluting the 50X HRP Conjugate in Antibody Diluent 4BI. To make 2 mL of the HRP Conjugate Solution combine 40 µL 50X HRP Conjugate with 1.96 mL Antibody Diluent 4BI. Mix thoroughly and gently.

Standard Preparation

Always prepare a fresh set of standards for every use. Discard working standard dilutions after use as they do not store well. The following section describes the preparation of a standard curve

for duplicate measurements (recommended).

1. Reconstitute the Human Cortisol standard sample by adding the volume of 1X Cell Extraction Buffer SSW indicated on the standard vial label. Hold at room temperature for 10 minutes. Mix thoroughly and gently. This is the 50,000 pg/mL **Stock Standard** Solution.
2. Label eight tubes, Standards 1– 8.
3. Add 288 µL of 1X Cell Extraction Buffer SSW into tube number 1 and 180 µL of 1X Cell Extraction Buffer SSW into numbers 2-8.
4. Use the **Stock Standard** to prepare the following dilution series. Standard #8 contains no standard and is the Blank control:

Standard #	Dilution Sample	Volume to Dilute (µL)	Volume of Diluent (µL)	Starting Conc. (pg/mL)	Final Conc. (pg/mL)
1	Stock Standard	72	288	50,000	10,000
2	Standard#1	180	180	10,000	5,000
3	Standard#2	180	180	5,000	2,500
4	Standard#3	180	180	2,500	1,250
5	Standard#4	180	180	1,250	625
6	Standard#5	180	180	625	312.5
7	Standard#6	180	180	312.5	156.3
8	Blank Control	0	180	N/A	N/A

Sample Preparation

Typical Sample Dynamic Range	
Sample Type	**Range**
Serum	0.31 – 2.5%
Plasma – Citrate	0.31 – 2.5%
Plasma – EDTA	0.31 – 2.5%
Plasma – Heparin	0.31 – 2.5%
Urine	0.16 – 2.5%
Cell Culture Supernatant	3.13 – 50%
Saliva	3.13 – 25%
Milk	3.13 – 12.5%

Serum Samples should be collected into a serum separator tube. After clot formation, centrifuge samples at 2,000 x g for 10 minutes and collect serum. Store un-diluted serum at -20°C or below. Avoid repeated freeze-thaw cycles.

Acid Treatment Protocol: For dissociation of free Cortisol in serum samples.

1) Add 50 µL of 1N HCL to 100 µL serum. Incubate at 15 minutes at room temperature while rotating.
2) Add 50 µL of 1N NaOH to acid treated sample. Incubate for 3 minutes at room temperature. The pH of the sample should be in the range of 6.0 – 7.5.
3) Serum concentration after treatment is 50%, dilute treated serum sample in Cell Extraction Buffer SSW to in-assay concentration (see typical dynamic range).

Plasma Collect plasma using citrate, EDTA or heparin. Centrifuge samples at 2,000 x g for 10 minutes. Store un-diluted plasma samples at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.

Acid Treatment Protocol: For dissociation of free Cortisol in plasma samples.

1) Add 50 µL of 1N HCL to 100 µL plasma. Incubate at 15 minutes at room temperature while rotating.
2) Add 50 µL of 1N NaOH to acid treated sample. Incubate for 3 minutes at room temperature. The pH of the sample should be in the range of 6.0 – 7.5.
3) Plasma concentration after treatment is 50%, dilute treated plasma sample in Cell Extraction Buffer SSW to in-assay concentration (see typical dynamic range).

Urine Centrifuge urine at 2,000 x g for 10 minutes to remove debris. Dilute samples at least 1:40 into Cell Extraction Buffer SSW and assay. Store un-diluted urine samples at -20°C or below. Avoid repeated freeze-thaw cycles.

Milk De-fat milk samples as follows. Centrifuge milk samples at 500 x g for 15 minutes at 4°C and collect the aqueous fraction using syringe attached to needle. Centrifuge the aqueous fraction at 3,000 x g for 15 minutes at 4°C and collect the final aqueous fraction (de-fatted milk) using syringe attached to needle. Store un-diluted de-fatted milk at -20°C or below. Avoid repeated freeze-thaw cycles.

Acid Treatment Protocol: For dissociation of free Cortisol in milk samples.

4) Add 50 µL of 1N HCL to 100 µL milk. Incubate at 15 minutes at room temperature while rotating.
5) Add 50 µL of 1N NaOH to acid treated sample. Incubate for 3 minutes at room temperature. The pH of the sample should be in the range of 6.0 – 7.5.
6) Plasma concentration after treatment is 50%, dilute treated milk sample in Cell Extraction Buffer SSW to in-assay concentration (see typical dynamic range).

Saliva Centrifuge saliva at 800 x g for 10 minutes to remove debris. Collect supernatants. Store un-diluted samples at -20°C or below. Avoid repeated freeze-thaw cycles.

Acid Treatment Protocol: For dissociation of free Cortisol in saliva samples.

7) Add 50 µL of 1N HCL to 100 µL saliva. Incubate at 15 minutes at room temperature while rotating.
8) Add 50 µL of 1N NaOH to acid treated sample. Incubate for 3 minutes at room temperature. The pH of the sample should be in the range of 6.0 – 7.5.
9) Plasma concentration after treatment is 50%, dilute treated saliva sample in Cell Extraction Buffer SSW to in-assay concentration (see typical dynamic range).

Cell Culture Supernatants Centrifuge cell culture media at 2,000 x g for 10 minutes to remove debris. Collect supernatants. Dilute samples into Cell Extraction Buffer SSW and assay. Store un-diluted samples at -20°C or below. Avoid repeated freeze-thaw cycles.

Plate Preparation

The 96 well plate strips included with this kit are supplied ready to use. It is not necessary to rinse the plate prior to adding reagents.

Unused plate strips should be immediately returned to the foil pouch containing the desiccant pack, resealed and stored at 4°C.

For each assay performed, a minimum of two wells must be used as the zero control.
For statistical reasons, we recommend each sample should be assayed with a minimum of two replicates (duplicates).
Differences in well absorbance or "edge effects" have not been observed with this assay.

Assay Procedure

Equilibrate all materials and prepared reagents to room temperature prior to use.
We recommend that you assay all standards, controls and samples in duplicate.

1. Prepare all reagents, working standards, and samples as directed in the previous sections.
2. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, reseal and return to 4°C storage.
3. Add 60 µL of all sample or standard to appropriate wells.
4. Add 30 µL of the CaptSure Conjugate Solution to each well.
    a. Optional – for non-specific binding wells, add 30 µL of Antibody Diluent 4BI in lieu of CaptSure Conjugate Solution.
5. Add 30 µL of the HRP Conjugate Solution to each well.
6. Seal the plate and incubate for 1 hour at room temperature on a plate shaker set to 400 rpm.
7. Wash each well with 3 x 350 µL 1X Wash Buffer PT. Wash by aspirating or decanting from wells then dispensing 350 µL 1X Wash Buffer PT into each well. Wash Buffer PT should remain in wells for at least 30 seconds. Complete removal of liquid at each step is essential for good performance. After the last wash invert the plate and tap gently against clean paper towels to remove excess liquid.
8. Add 100 µL of TMB Development Solution to each well and incubate for 10 minutes in the dark on a plate shaker set to 400 rpm.
   Given variability in laboratory environmental conditions, optimal incubation time may vary between 5 and 20 minutes.
   Note: The addition of Stop Solution will change the color from blue to yellow and enhance the signal intensity about 3X. To avoid signal saturation, proceed to the next step before the high concentration of the standard reaches a blue color of O.D.600 equal to 1.0.
9. Add 100 µL of Stop Solution to each well. Shake plate on a plate shaker for 1 minute to mix. Record the OD at 450 nm. This is an endpoint reading.
10. Alternative to 8 – 9: Instead of the endpoint reading at 450 nm, record the development of TMB Substrate kinetically. Immediately after addition of TMB Development Solution begin recording the blue color development with elapsed time in the microplate reader prepared with the following settings:

Mode	Kinetic
Wavelength:	600 nm
Time:	up to 20 min
Interval:	20 sec - 1 min
Shaking:	Shake between readings

Note that an endpoint reading can also be recorded at the completion of the kinetic read by adding 100 µL Stop Solution to each well and recording the OD at 450 nm.

Download our ELISA guide for technical hints, results, calculation, and troubleshooting tips:
www.abcam.com/protocols/the-complete-elisa-guide

For technical support contact information, visit: www.abcam.com/contactus

Version 1 | 2025-06-01

ab324547 – Human Cortisol SimpleStep ELISA® Kit – Extracellular (Hydrocortisone)

Additional information

ASSAY SPECIFICITY

This kit is designed for the quantification of Cortisol in Human samples.

The standard in this kit is free Cortisol

Native signal was detected in serum, plasma (citrate), plasma (EDTA), plasma (heparin), saliva, urine, and milk.

Spiked experiments were used to validate cell culture supernatant.

Cell and tissue extract samples have not been tested with this kit.

This kit is incompatible with mouse and rat samples.

For the measurement of Human Cortisol cell and tissue extracts, use Human Cortisol ELISA Kit - Intracellular ab324548.

CROSS REACTIVITY

Cross reactivity was determined for related compounds at 10,000 pg/mL and 100,000 pg/mL. Cross reactivity is reported as interpolated concentration relative to Cortisol.

Compound	Cross Reactivity – 10,000 pg/mL (%)	Cross Reactivity – 100,000 pg/mL (%)
Corticosterone	68	-
Prednisolone	19	-
Dexamethasone	8	63
Progesterone	7	59
Androstenedione	5	35
Cortisone	2	9
Aldosterone	0	6
Dihydrotestosterone	0	3
Estriol	0	3
3-CMO Testosterone	0	0
11a-Hydroxyprogesterone	0	0
17-β Estradiol	0	0
Androsterone	0	0
Danazol	0	0
Dehydroepiandrosterone (DHEA)	0	0
Dehydroepiandrosterone Sulfate (DHEA-S)	0	0
Estrone-3-Sulfate	0	0
Prednisone	0	0
Spironolactone	0	0
Tetrahydroxy-11-Deoxycortisol	0	0

INTERFERENCE

The above compounds were tested at 10,000 and 100,000 pg/mL for interference with 2,500 pg/mL of Cortisol. No interference was observed.

SPECIES REACTIVITY

Other species reactivity not determined.

CALCULATION

- Optional: Non-specific binding (NSB) well subtracted values can be calculated by averaging the absorbance values for the NSB wells and subtracting the average NSB absorbance value from all other absorbance values.
- Create a standard curve by plotting the average absorbance value for each standard concentration (y-axis) against the target concentration (x-axis) of the standard. Use graphing software to draw the best smooth curve through these points to construct the standard curve.
- Note: Most microplate reader software or graphing software will plot these values and fit a curve to the data. A four parameter curve fit (4PL) is often the best choice; however, other algorithms (e.g. linear, semi-log, log/log, 4 parameter logistic) can also be tested to determine if it provides a better curve fit to the standard values.
- Determine the concentration of the target analyte in the sample by interpolating absorbance values against the standard curve. Multiply the resulting value by the appropriate sample dilution factor, if used, to obtain the concentration of target analyte in the sample.
- Samples generating absorbance values lower than that of the highest concentration standard should be further diluted and reanalyzed. Similarly, samples which measure at an absorbance value greater than that of the lowest concentration standard should be retested in a less dilute form.
- Optional: The binding percentage, B/B_0, can be calculated by dividing the average absorbance value for each standard or sample by the average absorbance of the zero standard (B_0).

TYPICAL DATA

Typical standard curve – data provided for demonstration purposes only. A new standard curve must be generated for each assay performed.

Standard Curve Measurements				
Concentration (pg/mL)	O.D. 450 nm		Mean O.D.	B/B_0 (%)
	1	2		
NSB	0.035	0.033	0.034	-
0 (B_0)	1.513	1.505	1.509	100%
156.3	1.219	1.243	1.231	82%
312.5	1.068	1.058	1.063	70%
625	0.840	0.779	0.809	54%
1,250	0.554	0.582	0.568	38%
2,500	0.352	0.358	0.355	24%
5,000	0.213	0.209	0.211	14%
10,000	0.121	0.137	0.129	9%

Table 1. Example of Human Cortisol standard curve in Cell Extraction Buffer SSW. The Human Cortisol standard curve was prepared as described in the Standard Preparation section. The table shows raw data values.

TYPICAL SAMPLE VALUES

Sensitivity:

The calculated minimal detectable dose (MDD) is 44.7 pg/mL. The MDD was determined by calculating the mean of zero standard replicates (n=24) and subtracting 2 standard deviations then extrapolating the corresponding concentration.

Recovery

3 concentrations of Human Cortisol were spiked in duplicate to the indicated biological matrix to evaluate signal recovery in the working range of the assay.

Sample Type	Average % Recovery	Range (%)
2.5% Serum	105	103 – 110
2.5% Plasma – Citrate	101	97 – 103
2.5% Plasma – EDTA	101	95 – 105
2.5% Plasma – Heparin	95	86 – 106
1:160 Urine	95	90 – 98
50% Cell Culture Media	87	83 – 94
12.5% Saliva	104	96 – 108
12.5% Milk	116	114 – 119

*Media is DMEM containing 0% fetal calf serum.

Linearity of Dilution

Linearity of dilution is determined based on interpolated values from the standard curve. Linearity of dilution defines a sample concentration interval in which interpolated target concentrations are directly proportional to sample dilution.

Native Human Cortisol was measured in the following biological samples in a 2-fold dilution series. Sample dilutions are made in Cell Extraction Buffer SSW.

Dilution Factor	Interpolated value	2.5% Human Serum	2.5% Human Plasma (Citrate)	2.5% Human Plasma (EDTA)	2.5% Human Plasma (Heparin)	2.5% Human Urine
Undiluted	pg/mL	2,910	1,914	2,358	3,727	5,949
	% Expected value	100	100	100	100	100
2	pg/mL	1,686	932	1,309	1,891	3,083
	% Expected value	116	97	111	101	104
4	pg/mL	786	468	597	910	1,464
	% Expected value	108	98	101	98	98
8	pg/mL	313	235	243	424	646
	% Expected value	86	98	82	91	87

Dilution Factor	Interpolated value	12.5% Human Milk	25% Human Saliva
Undiluted	pg/mL	1,198	1,374
	% Expected value	100	100
2	pg/mL	491	646
	% Expected value	82	94
4	pg/mL	241	331
	% Expected value	80	96
8	pg/mL	ND	162
	% Expected value	ND	95

ND – Not Detected – below product dynamic range

Human Cortisol was spiked in in the following biological samples in a 2-fold dilution series. Sample dilutions are made in Cell Extraction Buffer SSW.

Dilution Factor	Interpolated value	50% Cell Culture Media
Undiluted	pg/mL	4,855
	% Expected value	100
2	pg/mL	2,513
	% Expected value	104
4	pg/mL	1,358
	% Expected value	112
8	pg/mL	684
	% Expected value	113
16	pg/mL	342
	% Expected value	113

Precision

Mean coefficient of variations of interpolated values of Human Cortisol from one concentration of Human serum within the working range of the assay.

	Intra-assay	Inter-assay
N=	8	3
CV (%)	3.3	1.3

Download our ELISA guide for technical hints, results, calculation, and troubleshooting tips:
www.abcam.com/protocols/the-complete-elisa-guide
For technical support contact information, visit: www.abcam.com/contactus

Version 1 | 2025-06-01