

ab325898 – Human HE4 SimpleStep ELISA® Kit, Chemiluminescent

For the quantitative measurement of HE4 in human serum, plasma (EDTA), plasma (citrate), cell culture supernatant, saliva, and urine.

For research use only - not intended for diagnostic use.

Patent pending.

For overview, typical data and additional information please visit: www.abcam.com/ab325898

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.

Limitations: All data, except typical standard curve and sensitivity were collected using the colorimetric version of this kit (ab240688).

Materials Supplied

Item	Quantity 1 x 96 tests	Storage Condition
Human HE4 Capture Antibody 10X	600 µL	+4°C
Human HE4 Detector Antibody 10X	600 µL	+4°C
Human HE4 Lyophilized Recombinant Protein	2 Vials	+4°C
Antibody Diluent 4BI	6 mL	+4°C
Cell Extraction Enhancer Solution 50X	1 mL	+4°C
Sample Diluent 25BS	20 mL	+4°C
Sample Diluent NS	50 mL	+4°C
Wash Buffer PT 10X	20 mL	+4°C
ChemiHRP Reagent A	3 mL	+4°C
ChemiHRP Reagent B	3 mL	+4°C
SimpleStep Pre-Coated Black 96-Well Microplate	96 Wells	+4°C
Plate Seal	1	+4°C

Materials Required, Not Supplied

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

Luminometer with the following settings: 0.5-1 second/well read time; summation mode (all wavelengths).

Deionized water.

Multi- and single-channel pipettes.

Tubes for standard dilution.

Orbital microplate shaker for all incubation steps: capable of 750 rpm shaking speed.

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. Capture and Detector Antibodies have only been tested for stability in the provided 10X formulations.

Sample Diluent 25BS may contain precipitate, this is normal. If precipitate is not dissolved by gentle mixing, the precipitate may be dissolved by gentle warming and mixing at 37°C for 10 minutes. If precipitate remains, gently spin down and avoid visible precipitates when pipetting.

The provided Cell Extraction Enhancer Solution 50X may precipitate when stored at + 4°C. To dissolve, warm briefly at + 37°C and mix gently. The Cell Extraction Enhancer Solution 50X can be stored at room temperature to avoid precipitation.

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.

Sample Diluent 25BS + 1X Enhancer:

Prepare Sample Diluent 25BS + 1X Enhancer by diluting 50X Cell Extraction Enhancer Solution to 1X in Sample Diluent 25BS. To make 5 mL Sample Diluent 25BS + 1X Enhancer, combine 4.9 mL Sample Diluent 25BS and 100 µL Cell Extraction Enhancer Solution 50X. Mix thoroughly and gently. If required protease inhibitors can be added.

Sample Diluent NS + 1X Enhancer: Prepare Sample Diluent NS + 1X Enhancer by combining Sample Diluent NS and 50X Cell Extraction Enhancer Solution. To make 10 mL Sample Diluent NS + 1X Enhancer, combine 9.8 mL Sample Diluent NS and 200 µL Cell Extraction Enhancer Solution 50X. Mix thoroughly and gently.

Antibody Cocktail: Prepare Antibody Cocktail by diluting the capture and detector antibodies in Antibody Diluent 4BI. To make 3 mL of the Antibody Cocktail combine 300 µL 10X Capture Antibody and 300 µL 10X Detector Antibody with 2.4 mL Antibody Diluent 4BI. Mix thoroughly and gently.

Lumi HRP Development Solution: Just prior to use, prepare Lumi HRP Development Solution by mixing equal volume of the ChemiHRP Reagent A and the ChemiHRP Reagent B. To make 3 mL of the Lumi HRP Development Solution combine 1.5 mL of ChemiHRP Reagent A and 1.5 mL of ChemiHRP Reagent B. Mix thoroughly and gently by inversion or slow pipetting (Avoid shaking or vortexing). Protect the prepared solution from light until use.

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 **HE4** protein standard by adding the volume indicated on the protein vial label. For **serum and plasma samples measurements**, use Sample Diluent 25BS + 1X Enhancer. For **saliva, urine and cell culture supernatant samples measurements**, use Sample Diluent NS + 1X Enhancer. Hold at room temperature for 10 minutes and mix thoroughly and gently. This is the 14.5 ng/mL **Stock Standard** Solution.
1. Label eight tubes, Standards 1– 8.
2. Use the same Sample Diluent as used to resuspend the Stock Standard to prepare the standard curve. Add 108 µL of Sample Diluent into tube number 1 and 150 µL of Sample Diluent into numbers 2-8.
3. Use the **Stock Standard** to prepare the following dilution series. Standard #8 contains no protein 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	240	108	14,500	10,000
2	Standard#1	75	150	10,000	3333
3	Standard#2	75	150	3333	1111
4	Standard#3	75	150	1111	370
5	Standard#4	75	150	370	123
6	Standard#5	75	150	123	41.2
7	Standard#6	75	150	41.2	13.7
8	Blank Control	0	150	0	0

Sample Preparation

Typical Sample Dynamic Range	
Sample Type	Range
Serum	0.8 – 25%
Plasma - Citrate	0.8 – 25%
Plasma - EDTA	0.8 – 25%
Saliva	1:32,000 – 1:1,000
Urine	1:32,000 – 1:1,000
OVCAR-3 cell culture supernatant	1:16,000 – 1:1,000

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. Dilute samples at least 1:4 into Sample Diluent 25BS + 1X Enhancer and assay. Store un-diluted serum at -20°C or below. Avoid repeated freeze-thaw cycles.

Plasma Collect plasma using citrate or EDTA. Centrifuge samples at 2,000 x g for 10 minutes. Dilute samples at least 1:4 into Sample Diluent 25BS + 1 X Enhancer and assay. Store un-diluted plasma samples at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles. Note: This kit is incompatible with plasma (heparin) samples.

Cell Culture Supernatants Centrifuge cell culture media at 2,000 x g for 10 minutes to remove debris. Collect supernatants. Dilute samples at least 1:1,000 into Sample Diluent NS + 1X Enhancer and assay. Store un-diluted samples at -20°C or below. Avoid repeated freeze-thaw cycles.

Urine Centrifuge urine at 2,000 x g for 10 minutes to remove debris. Dilute samples at least 1:1,000 into Sample Diluent NS + 1X Enhancer and assay. Store un-diluted urine samples at -20°C or below. Avoid repeated freeze-thaw cycles.

Saliva Centrifuge saliva at 800 x g for 10 minutes to remove debris. Collect supernatants. Dilute samples at least 1:1,000 into Sample Diluent NS + 1X Enhancer 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 50 µL of all sample or standard to appropriate wells.
4. Add 50 µL of the Antibody Cocktail to each well.
5. Seal the plate and incubate for 30 minutes at room temperature on a plate shaker set to 750 rpm.
6. 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.
7. Add 50 µL of prepared Lumi HRP Development Solution to each well and incubate for 1 minute in the dark on a plate shaker set to 750 rpm. Further optimization of incubation time vs signal strength can be performed if needed. Avoid introducing bubbles into the wells.
8. Measure the produced light of each well using a microplate luminometer with the following settings: 0.5-1 second/well read time in summation mode (all wavelengths). Relative light unit (RLU) readings may vary between luminometer models. It is recommended to configure instrument settings according to the manufacturer's specifications. Note: Relative light unit (RLU) values may change over the course of the 15-minute reading window.
9. Analyze the data as described below.

Mode:	Luminescence
Instrument settings:	Endpoint
Detection Mode:	All wavelengths
Read Time:	0.5-1 sec
Read:	Top

Note For microplate readers with Pre-Read Optimization option, the Read Height as well as Microplate Optimization is recommended before the first read.

Download our ELISA guide for technical hints, results, calculation, and troubleshooting tips:

<https://www.abcam.com/en-us/technical-resources/guides/elisa-guide>

Technical Support

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Version 1a | 2026-02-16

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Additional information

ASSAY SPECIFICITY

This kit is designed for the quantification of human HE4.

The standard protein in this kit is full-length mature human HE4.

Native signal was detected in serum, plasma (citrate), plasma (EDTA,) cell culture supernatant, urine, and saliva sample types.

Milk, CSF, and cell and tissue extract samples have not been tested with this kit.

This kit is incompatible with plasma (heparin) samples.

SPECIES REACTIVITY

Other species reactivity was determined by measuring 25% serum samples of various species, interpolating the protein concentrations from the human standard curve, and expressing the interpolated concentrations as a percentage of the protein concentration in human serum assayed at the same dilution.

Reactivity was determined to be < 3% for the following species: Mouse, Rat, Cow

Other species reactivity not determined.

CALCULATION

- Preconfigured protocols are available when using SoftMax Pro software from Molecular Devices.
- Calculate the average chemiluminescence value for the blank control (zero) standards. Subtract the average blank control standard chemiluminescence value from all other chemiluminescence values.
- Create a standard curve by plotting the average blank control subtracted chemiluminescence value for each standard concentration (y-axis) against the target protein 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 chemiluminescence 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 protein in the sample by interpolating the blank control subtracted chemiluminescence values against the standard curve. Multiply the resulting value by the appropriate sample dilution factor, if used, to obtain the concentration of target protein in the sample.
- Samples generating chemiluminescence values greater than that of the highest standard should be further diluted and reanalyzed. Similarly, samples which measure at chemiluminescence values less than that of the lowest standard should be retested in a less dilute form.

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)	RLU		Mean RLU
	1	2	
0	7,802	7,805	7,804
13.7	10,952	9,580	10,266
41.1	14,226	12,888	13,557
123	23,652	22,745	23,199
370	61,288	63,020	62,154
1,111	192,640	208,970	200,805
3,333	619,580	604,840	612,210
10,000	1,987,800	1,953,000	1,970,400

Table 1. Example of human HE4 standard curve in Sample Diluent 25BS + 1X Enhancer. The HE4 standard curve was prepared as described in the Standard Preparation section. The table shows raw data values.

Standard Curve Measurements			
Concentration (pg/mL)	RLU		Mean RLU
	1	2	
0	5,352	7,538	6,445
13.7	9,576	9,650	9,613
41.1	15,099	14,430	14,765
123	31,411	31,929	31,670
370	86,187	89,722	87,955
1,111	260,320	264,350	262,335
3,333	877,350	884,780	881,065
10,000	2,464,200	2,386,100	2,425,150

Table 2. Example of human HE4 standard curve in Sample Diluent NS + 1X Enhancer. The HE4 standard curve was prepared as described in the Standard Preparation section. The table shows raw data values.

TYPICAL SAMPLE VALUES

Sensitivity:

The minimal detectable dose (MDD) was determined by calculating the mean of zero standard replicates and adding 2 standard deviations then extrapolating the corresponding concentration.

Sample Diluent Buffer	N=	Minimal Detectable Dose
Sample Diluent 25BS + 1X Enhancer	16	13.39 pg/mL
Sample Diluent NS + 1X Enhancer	16	7.61 pg/mL

Recovery

Three concentrations of HE4 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 (%)
Serum (6.3%)	108	99 - 116
Plasma – Citrate (6.3%)	100	88 - 115
Plasma – EDTA (6.3%)	115	111 - 119
Saliva (1:4,000)	118	111 - 123
Urine (1:4,000)	108	106 - 111
OVCAR-3 Cell Culture Supernatant (1:2,000)	106	98 - 112

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 HE4 was measured in the following biological samples in a 2-fold dilution series. Sample dilutions are made in Sample Diluent 25BS + 1X Enhancer.

Dilution Factor	Interpolated value	25% Human Serum	25% Human Plasma (Citrate)	25% Human Plasma (EDTA)
Undiluted	pg/mL	1,595	1,243	1,337
	% Expected value	100	100	100
2	pg/mL	747	666	726
	% Expected value	94	107	109
4	pg/mL	359	336	357
	% Expected value	90	108	107
8	pg/mL	164	154	161
	% Expected value	82	99	96
16	pg/mL	79	71	67
	% Expected value	84	91	81

Native HE4 was measured in the following biological samples in a 2-fold dilution series. Sample dilutions are made in Sample Diluent NS + 1X Enhancer.

Dilution Factor	Interpolated value	1: 1,000 Human Saliva	1: 1,000 Human Urine	1: 1,000 OVCAR-3 Supernatant
Undiluted	pg/mL	1,463	1,288	520
	% Expected value	100	100	100
2	pg/mL	737	623	253
	% Expected value	101	97	97
4	pg/mL	351	303	129
	% Expected value	96	94	99
8	pg/mL	167	152	62
	% Expected value	91	94	95
16	pg/mL	88	78	38
	% Expected value	96	97	117

Precision

Mean coefficient of variations of interpolated values of HE4 from three concentrations of OVCAR-3 cell culture supernatant within the working range of the assay.

	Intra-assay	Inter-assay
N=	5	3
CV (%)	3.9	6.2

Download our ELISA guide for technical hints, results, calculation, and troubleshooting tips:

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