ab326897 – Rat Erythropoietin SimpleStep ELISA® Kit (EPO) – Chemiluminescent

For the quantitative measurement of Erythropoietin in rat serum, plasma (heparin), plasma (EDTA), plasma (citrate), and cell culture supernatant.
For research use only - not intended for diagnostic use.
Patent pending.

For overview, typical data and additional information please visit: www.abcam.com/ab326897

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 (plus any sample up-validation, native linearity, or individual donors testing results if available) were collected using the colorimetric version of this kit (ab274398).

Materials Supplied

Item	Quantity 1 x 96 tests	Storage Condition
Rat Erythropoietin Capture Antibody 10X	600 µL	+4°C
Rat Erythropoietin Detector Antibody 10X	600 µL	+4°C
Rat Erythropoietin Lyophilized Recombinant Protein	2 Vials	+4°C
Antibody Diluent CPR2	6 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.

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.

Antibody Cocktail: Prepare Antibody Cocktail by diluting the capture and detector antibodies in Antibody Diluent CPR2. 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 CPR2. 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 Erythropoietin standard sample by adding the volume of Sample Diluent NS indicated on the protein vial label. Hold at room temperature for 10 minutes. Mix thoroughly and gently. This is the 22,400 pg/mL **Stock Standard** Solution.
2. Label nine tubes, Standards 1– 9.
3. Add 149 µL of Sample Diluent NS into tube number 1 and 150 µL of Sample Diluent NS into numbers 2-9.
4. Use the **Stock Standard** to prepare the following dilution series. Standard #9 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**	150	298	22,400	7,500
2	Standard#1	75	150	7,500	2,500
3	Standard#2	75	150	2,500	833.3
4	Standard#3	75	150	833.3	277.8
5	Standard#4	75	150	277.8	92.59
6	Standard#5	75	150	92.59	30.86
7	Standard#6	75	150	30.86	10.29
8	Standard#7	75	150	10.29	3.43
9	Blank Control	0	150	0	0

Sample Preparation

Typical Sample Dynamic Range	
Sample Type	**Range**
Serum	12.5 – 100%
Plasma - Citrate	12.5 – 100%
Plasma - EDTA	25 – 100%
Plasma - Heparin	25 – 100%
Cell Culture Supernatant*	<100%

*Based on spiked sample

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. Assay, or dilute samples into Sample Diluent NS and assay. Store un-diluted serum at -20°C or below. Avoid repeated freeze-thaw cycles.
Plasma Collect plasma using citrate, EDTA or heparin. Centrifuge samples at 2,000 x g for 10 minutes. Assay, or dilute samples into Sample Diluent NS and assay. Store un-diluted plasma samples at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.
Cell Culture Supernatants Centrifuge cell culture media at 2,000 x g for 10 minutes to remove debris. Collect supernatants. Assay, or dilute samples into Sample Diluent NS 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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For all technical or commercial enquiries please go to:
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Additional information

ASSAY SPECIFICITY
This kit is designed for the quantification of rat Erythropoietin.

The standard protein in this kit is rat Erythropoietin.

Native signal was detected in serum, plasma (citrate), plasma (EDTA), and plasma (heparin) sample types.

Spiked protein experiments were used to validate cell culture supernatant sample types.

Urine, saliva, milk, CSF, cell extract, and tissue extract samples have not been tested with this kit.

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

Reactivity was determined for the following species: Human (43%), Monkey (92%), Mouse (135%).

Reactivity < 3% was determined for the following species: 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	50	50	50
3.43	1,554	1,758	1,656
10.29	5,626	6,080	5,853
30.86	18,032	22,791	20,412
92.59	78,819	89,377	84,098
277.8	190,864	200,117	195,491
833.3	681,848	651,118	666,483
2,500	1,801,384	1,932,235	1,866,810
7,500	4,374,738	4,774,526	4,574,632

Table 1. Example of rat Erythropoietin standard curve in Sample Diluent NS. The Erythropoietin 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 0.55 pg/mL. The MDD was determined by calculating the mean of zero standard replicates (n=15) and adding 2 standard deviations then extrapolating the corresponding concentration.

Recovery
Three concentrations of Erythropoietin recombinant protein 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 (%)
100% Serum	105	101 - 109
100% Plasma - Citrate	89	81 - 96
100% Plasma - EDTA	92	88 - 97
100% Plasma - Heparin	87	81 - 91
100% Cell Culture Media*	93	92 - 95

*Media is RPMI 1640 containing 10% fetal bovine 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 Erythropoietin was measured in the following Sprague Dawley (SD), Wistar and Fischer rat biological samples in a 2-fold dilution series. Sample dilutions are made in Sample Diluent NS.

Dilution Factor	Interpolated value	100% SD Rat Serum	100% SD Rat Plasma (Citrate)	100% SD Rat Plasma (EDTA)	100% SD Rat Plasma (Heparin)
Undiluted	pg/mL	124	93.2	43.6	72.7
	% Expected value	100	100	100	100
2	pg/mL	66.5	47.7	22.8	37.7
	% Expected value	107	102	105	104
4	pg/mL	35.4	25.6	11.3	17.9
	% Expected value	114	110	104	98
8	pg/mL	17.8	12.8	ND	ND
	% Expected value	115	110	ND	ND

ND – Not Detected – below product dynamic range

Dilution Factor	Interpolated value	100% Wistar Rat Serum	100% Fischer Rat Serum
Undiluted	pg/mL	97.3	60.5
	% Expected value	100	100
2	pg/mL	53.2	32.8
	% Expected value	109	108
4	pg/mL	28.8	16.2
	% Expected value	118	107
8	pg/mL	14.3	ND
	% Expected value	117	ND

Recombinant Erythropoietin was spiked into the following biological samples and diluted in a 2-fold dilution series in Sample Diluent NS.

Dilution Factor	Interpolated value	100% SD Rat Serum	100% SD Rat Plasma (Citrate)	100% SD Rat Plasma (EDTA)	100% SD Rat Plasma (Heparin)	100% Cell Culture Media
Undiluted	pg/mL	584	562	502	559	451
	% Expected value	100	100	100	100	100
2	pg/mL	318	311	271	293	242
	% Expected value	109	111	108	105	107
4	pg/mL	158	158	135	147	120
	% Expected value	108	112	108	105	107
8	pg/mL	85.5	80.0	63.4	78.9	60.4
	% Expected value	117	114	101	113	107
16	pg/mL	41.0	36.7	31.8	37.5	31.2
	% Expected value	112	104	101	107	111

Precision

Mean coefficient of variations of interpolated values of Erythropoietin from two concentrations of recombinant rat Erythopoietin spiked into neat cell culture media within the working range of the assay.

	Intra-assay	Inter-assay
n=	8	3
CV (%)	4.2	3.5

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