ab325725 – Human Polymeric immunoglobulin receptor SimpleStep ELISA® Kit (PIGR), Chemiluminescent

For the quantitative measurement of Polymeric immunoglobulin receptor in human serum, plasma (heparin), plasma (EDTA), plasma (citrate), cell culture supernatant, and cell and tissue extract.
For research use only - not intended for diagnostic use.
Patent pending.

For overview, typical data and additional information please visit: www.abcam.com/ab325725

Storage and Stability: Store kit at 2-8ºC 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 (ab282302).

Materials Supplied

Item	Quantity 1 x 96 tests	Storage Condition
Human Polymeric immunoglobulin receptor Capture Antibody 10X	600 µL	+4ºC
Human Polymeric immunoglobulin receptor Detector Antibody 10X	600 µL	+4ºC
Human Polymeric immunoglobulin receptor Lyophilized Recombinant Protein	2 Vials	+4ºC
Antibody Diluent 5BI	6 mL	+4ºC
Cell Extraction Buffer PTR 5X	10 mL	+4ºC
Sample Diluent NS	50 mL	+4ºC
Wash Buffer PT 10X	20 mL	+4ºC
ChemiHRP Reagent A	3 mL	+4ºC
ChemiHRP Reagent B	3 mL	+4ºC
SimpleStep Pre-Coated Black 96-Well Microplate	96 Wells	+4ºC
Plate Seal	1	+4ºC

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).
Method for determining protein concentration (BCA assay recommended).
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 (18-25ºC) 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.

1X Cell Extraction Buffer PTR (For cell and tissue extracts only): Prepare 1X Cell Extraction Buffer PTR by diluting Cell Extraction Buffer PTR 5X to 1X with deionized water. To make 10 mL 1X Cell Extraction Buffer PTR combine 8 mL deionized water and 2 mL Cell Extraction Buffer PTR 5X. Mix thoroughly and gently. If required protease inhibitors can be added.

Antibody Cocktail: Prepare Antibody Cocktail by diluting the capture and detector antibodies in Antibody Diluent 5BI. 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 5BI. 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 **Polymeric immunoglobulin receptor** protein standard by adding the volume indicated on the protein vial label. For **serum, plasma and cell culture supernatant samples**, use Sample Diluent NS. For **cell extract and tissue extract samples measurements**, use 1X Cell Extraction Buffer PTR. Hold at room temperature for 10 minutes and mix thoroughly and gently. This is the 80 ng/mL **Stock Standard #1** Solution.
2. Label eight tubes, Standards 1– 8.
3. Use the same Sample Diluent as used to resuspend the Stock Standard #1 to prepare the standard curve. Add 150 µL of Sample Diluent into numbers 2-8.
4. Use the **Stock Standard #1** 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 #1**	150	0	80,000	80,000

2	**Stock Standard #1**	75	150	80,000	26,667
3	Standard#2	75	150	26,667	8,889
4	Standard#3	75	150	8,889	2,963
5	Standard#4	75	150	2,963	988
6	Standard#5	75	150	988	329
7	Standard#6	75	150	329	110
8	Blank Control	0	150	0	0

Sample Preparation

Typical Sample Dynamic Range	
Sample Type	**Range**
Serum	0.039 - 1.25%
Plasma – Citrate	0.039 - 1.25%
Plasma – EDTA	0.039 - 1.25%
Plasma – Heparin	0.039 - 1.25%
Cell Culture Media*	≤ 50%
Stomach Tissue Extract	31.25 - 500 μg/mL

*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. Dilute samples at least 1:80 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. Dilute samples at least 1:80 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 and assay. Or dilute samples at least 1:2 into Sample Diluent NS and assay. Store un-diluted samples at -20°C or below. Avoid repeated freeze-thaw cycles.

Preparation of extracts from cell pellets Collect non-adherent cells by centrifugation or scrape to collect adherent cells from the culture flask. Typical centrifugation conditions for cells are 500 x g for 5 minutes at 4°C. Rinse cells twice with PBS. Solubilize pellet at 2x10^7 cell/mL in chilled 1X Cell Extraction Buffer PTR. Incubate on ice for 20 minutes. Centrifuge at 18,000 x g for 20 minutes at 4°C. Transfer the supernatants into clean tubes and discard the pellets. Assay samples immediately or aliquot and store at -80°C. The sample protein concentration in the extract may be quantified using a protein assay. Dilute samples to desired concentration in 1X Cell Extraction Buffer PTR.

Preparation of extracts from adherent cells by direct lysis (alternative protocol) Remove growth media and rinse adherent cells 2 times in PBS. Solubilize the cells by addition of chilled 1X Cell Extration Buffer PTR directly to the plate (use 750 µL - 1.5 mL 1X Cell Extration Buffer PTR per confluent 15 cm diameter plate). Scrape the cells into a microfuge tube and incubate the lysate on ice for 15 minutes. Centrifuge at 18,000 x g for 20 minutes at 4°C. Transfer the supernatants into clean tubes and discard the pellets. Assay samples immediately or aliquot and store at -80°C. The sample protein concentration in the extract may be quantified using a protein assay. Dilute samples to desired concentration in 1X Cell Extration Buffer PTR.

Preparation of extracts from tissue homogenates Tissue lysates are typically prepared by homogenization of tissue that is first minced and thoroughly rinsed in PBS to remove blood (dounce homogenizer recommended). Homogenize 100 to 200 mg of wet tissue in 500 µL – 1 mL of chilled 1X Cell Extration Buffer PTR. For lower amounts of tissue adjust volumes accordingly. Incubate on ice for 20 minutes. Centrifuge at 18,000 x g for 20 minutes at 4°C. Transfer the supernatants into clean tubes and discard the pellets. Assay samples immediately or aliquot and store at -80°C. The sample protein concentration in the extract may be quantified using a protein assay. Dilute samples to desired concentration in 1X Cell Extration Buffer PTR.

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:
https://www.abcam.com/en-us/contact-us
https://www.abcam.cn/contact-us (China)
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Additional information

ASSAY SPECIFICITY

The standard protein in this kit is full-length secretory component of human Polymeric immunoglobulin receptor.

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

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

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

SPECIES REACTIVITY

Other species reactivity was determined by measuring 1.25% 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 < 3% was determined for the following species: Monkey, 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	10,677	10,677	10,677
110	15,655	15,645	15,650
329	24,897	24,709	24,803
988	50,064	49,969	50,017
2,963	125,330	131,080	128,205
8,889	376,850	365,590	371,220
26,667	1,175,400	1,127,700	1,151,550
80,000	3,582,300	3,285,000	3,433,650

Table 1. Example of human Polymeric immunoglobulin receptor standard curve in Sample Diluent NS. The Polymeric immunoglobulin receptor 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	11,083	11,083	11,083
110	15,476	13,977	14,727
329	23,228	21,267	22,248
988	48,986	48,138	48,562
2,963	123,690	119,030	121,360
8,889	332,210	363,960	348,085
26,667	1,020,800	989,460	1,005,130
80,000	3,507,300	3,412,700	3,460,000

Table 2. Example of human Polymeric immunoglobulin receptor standard curve in 1X Cell Extration Buffer PTR. The Polymeric immunoglobulin receptor 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 NS	16	40.9 pg/mL
1X Cell Extraction Buffer PTR	16	54.9 pg/mL

Recovery

Three concentrations of Polymeric immunoglobulin receptor 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 (%)
0.156% Serum	85	82 - 87
0.156% Plasma – Citrate	90	81 - 98
0.156% Plasma – EDTA	83	80 - 87
0.156% Plasma – Heparin	97	81 - 92
50% Cell Culture Media*	87	85 - 89
125 µg/mL Stomach Tissue Extract	112	108 - 117

*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 Polymeric immunoglobulin receptor was measured in the following biological samples in a 2-fold dilution series. Sample dilutions are made in Sample Diluent NS.

Dilution Factor	Interpolated value	1.25% Serum	1.25% Plasma (Citrate)	1.25% Plasma (EDTA)	1.25% Plasma (Heparin)
Undiluted	pg/mL	7,353.89	8,907.13	9,726.49	8,240.52
	% Expected value	100	100	100	100
2	pg/mL	3,674.85	4,312.1	4,873.17	4,062.66
	% Expected value	100	97	100	99
4	pg/mL	1,954.80	2,238.90	2,434.73	2,126.86
	% Expected value	106	101	100	103
8	pg/mL	997.10	1,091.62	1,181.33	1,047.90
	% Expected value	108	98	97	102
16	pg/mL	489.15	586.47	669.13	523.54
	% Expected value	106	105	110	102

Recombinant Polymeric immunoglobulin receptor was spiked into the following biological samples and then diluted in a 2-fold dilution series. Sample dilutions are made in Sample Diluent NS.

Dilution Factor	Interpolated value	50% Cell Culture Media*
Undiluted	pg/mL	4,362.75
	% Expected value	100
2	pg/mL	2,295.42
	% Expected value	105
4	pg/mL	1,198.27
	% Expected value	110
8	pg/mL	648.77
	% Expected value	119

*Media is RPMI 1640 containing 10% fetal bovine serum.

Native Polymeric immunoglobulin receptor was measured in the following biological samples in a 2-fold dilution series. Sample dilutions are made in 1X Cell Extraction Buffer PTR.

Dilution Factor	Interpolated value	500 µg/mL Stomach Tissue Extract
Undiluted	pg/mL	11,148.31
	% Expected value	100
2	pg/mL	5,690.44
	% Expected value	102
4	pg/mL	2,723.50
	% Expected value	98
8	pg/mL	1,453.96
	% Expected value	104
16	pg/mL	673.48
	% Expected value	97

Precision

Mean coefficient of variations of interpolated values of Polymeric immunoglobulin receptor from a single concentration of human serum within the working range of the assay.

	Intra-assay	Inter-assay
N=	8	3
CV (%)	3.3	5.0

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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