

ab288179 – Human IRAK4 CatchPoint® SimpleStep ELISA® Kit

For the quantitative measurement of IRAK4 in human cell and tissue extract samples
For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit: www.abcam.com/ab288179

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 (ab213472).

Materials Supplied

Item	Quantity	Storage Condition
Human IRAK4 Capture Antibody 10X	600 µL	+4°C
Human IRAK4 Detector Antibody 10X	600 µL	+4°C
Human IRAK4 Lyophilized Recombinant Protein	2 Vials	+4°C
Antibody Diluent 4BC	6 mL	+4°C
Cell Extraction Buffer PTR 5X	10 mL	+4°C
Sample Diluent NS	12 mL	+4°C
Wash Buffer PT 10X	20 mL	+4°C
Stoplight Red Substrate Buffer	12 mL	+4°C
100X Stoplight Red Substrate	120 µL	+4°C
500X Hydrogen Peroxide (H ₂ O ₂ , 3%)	50 µL	+4°C
Sample Diluent NS	12 mL	+4°C
SimpleStep Pre-Coated Black 96-Well Microplate	96 Wells	+4°C
Plate Seal	1	+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:

Fluorescence microplate reader Ex/Cutoff/Em 530/570/590 nm.

Method for determining protein concentration (BCA assay recommended).

Deionized water.

Multi- and single-channel pipettes.

Tubes for standard dilution.

Plate shaker for all incubation steps.

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 Cell Extraction Buffer PTR (For cell and tissue extracts only): Prepare 1X Cell Extraction Buffer PTR by diluting Cell Extraction Buffer PTR 5X and 50X Cell Extraction Enhancer Solution 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.

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 4BC. 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 4BC. Mix thoroughly and gently.

CatchPoint HRP Development Solution: Just prior to use, prepare CatchPoint HRP Development Solution by diluting the 100X Stoplight Red Substrate and the 500X Hydrogen Peroxide in Stoplight Red Substrate Buffer. For example, to make 6 mL of the CatchPoint HRP Development Solution combine 60 µL 100X Stoplight Red Substrate and 12 µL of 500X Hydrogen Peroxide with 5.928 mL Stoplight Red Substrate Buffer. 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).

- IMPORTANT:** If the protein standard vial has a volume identified on the label, reconstitute the IRAK4 standard by adding that volume of 1X Cell Extraction Buffer PTR indicated on the label. Alternatively, if the vial has a mass identified, reconstitute the IRAK4 standard by adding 500 µL 1X Cell Extraction Buffer PTR. Hold at room temperature for 10 minutes and mix gently. This is the 80 ng/mL **Stock Standard** Solution.
- Label eight tubes, Standards 1–8.
- Add 60 µL of 1X Cell Extraction Buffer PTR into tube number 1 and 150 µL of 1X Cell Extraction Buffer PTR into numbers 2-8.
- 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. (ng/mL)	Final Conc. (ng/mL)
1	Stock Standard	240	60	80	64
2	Standard#1	150	150	64	32
3	Standard#2	150	150	32	16
4	Standard#3	150	150	16	8

5	Standard#4	150	150	8	4
6	Standard#5	150	150	4	2
7	Standard#6	150	150	2	1
8	Standard#7	150	150	1	0.50
9	Standard#8	150	150	0.50	0.25
10	Blank Control	0	150	n/a	0

Sample Preparation

Typical Sample Dynamic Range	
Sample Type	Range
THP1 Cell Extract	31.25 – 500 µg/mL
PBMC Cell Extract	15.63 – 500 µg/mL
Human Placenta Tissue Homogenate Extract	15.63 – 500 µg/mL

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 2x10⁷ 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 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 Extraction 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 Extraction 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 1 hour at room temperature on a plate shaker set to 400 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 10 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 100 µL of prepared CatchPoint HRP Development Solution to each well and incubate for 10 minutes in the dark on a plate shaker set to 400 rpm. Further optimization of incubation time vs signal strength can be performed if needed.
8. Record the fluorescence at Ex/Cutoff/Em 530/570/590 nm. If using a Molecular Devices' plate reader supported by SoftMax® Pro software, a preconfigured protocol for these CatchPoint SimpleStep ELISA Kits is available with all the protocol and analysis settings at www.softmaxpro.org.
9. Analyze the data as described below.

Mode	Fluorescence
Instrument settings:	Endpoint
Excitation:	530 nm
Cutoff:	570 nm
Emission:	590 nm
Sensitivity:	6 flashes/read or 200ms
PMT:	Auto
Auto calibrate:	On
Read:	Top
Read Height:	1*

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:

www.abcam.com/protocols/the-complete-elisa-guide

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

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Additional information

ASSAY SPECIFICITY

This kit is designed for the quantification of human IRAK4.

Native signal was detected in cell and tissue extract samples.

Serum, plasma (heparin), plasma (EDTA), plasma (citrate), cell culture supernatant, saliva, urine, milk, and cerebrospinal fluid samples have not been tested with this kit.

CALCULATION

- Preconfigured protocols are available when using SoftMax Pro software from Molecular Devices
- Calculate the average fluorescence value for the blank control (zero) standards. Subtract the average blank control standard fluorescence value from all other fluorescence values.
- Create a standard curve by plotting the average blank control subtracted fluorescence 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 fluorescence 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 fluorescence 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 fluorescence values greater than that of the highest standard should be further diluted and reanalyzed. Similarly, samples which measure at fluorescence 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 (ng/mL)	RFU (x 10 ⁶)		Mean RFU
	1	2	
0	0.827	0.746	0.787
0.25	1.698	1.668	1.683
0.50	2.339	2.591	2.465
1	4.295	5.186	4.740
2	6.586	9.377	7.982
4	16.531	19.270	17.900
8	33.150	36.963	35.057
16	67.293	73.886	70.590
32	101.372	127.480	114.426
64	176.197	215.430	195.813

Table 1. Example of human IRAK4 standard curve in 1X Cell Extraction Buffer PTR. The IRAK4 standard curve was prepared as described in the Standard Preparation section. The table shows raw data values generated on Molecular Devices SpectraMax iD3 Multi-Mode Microplate reader.

TYPICAL SAMPLE VALUES

Sensitivity:

The calculated minimal detectable dose (MDD) is 0.137 ng/mL. The MDD was determined by calculating the mean of zero standard replicates (n=22) and adding 2 standard deviations then extrapolating the corresponding concentration.

Recovery

Three concentrations of IRAK4 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 (%)
THP1 Cell Extract (30 µg/mL)	111	107 – 115
PBMC Cell Extract (30 µg/mL)	102	101 – 102
Human Placenta Homogenate (300 µg/mL)	101	100-101

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 IRAK4 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 THP1 Cell Extract	500 µg/mL PBMC Cell Extract
Undiluted	ng/mL	40.76	40.67
	% Expected value	100	100
2	ng/mL	23.81	24.45
	% Expected value	117	120
4	ng/mL	10.66	11.65
	% Expected value	105	115
8	ng/mL	4.83	5.60
	% Expected value	95	110
16	ng/mL	2.06	2.80
	% Expected value	81	110

Recombinant IRAK4 was spiked in in the following biological samples in a 2-fold dilution series. Sample dilutions are made in Sample Diluent PTR.

Dilution Factor	Interpolated value	500 µg/mL Human Placenta Homogenate Extract
Undiluted	ng/mL	23.71
	% Expected value	100
2	ng/mL	10.69
	% Expected value	90
4	ng/mL	5.05
	% Expected value	85
8	ng/mL	2.38
	% Expected value	80
16	ng/mL	1.34
	% Expected value	90

Precision

Mean coefficient of variations of interpolated values of IRAK4 from three concentrations of THP1 extract within the working range of the assay.

	Intra-assay	Inter-assay
N=	8	3
CV (%)	5.0	4.0

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

www.abcam.com/protocols/the-complete-elisa-guide

Technical Support

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