

## ab284016 – Human PAK4 ELISA Kit

For the quantitative measurement of PAK4 in human plasma, serum and cell culture supernatant samples.

For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit: [www.abcam.com/ab284016](http://www.abcam.com/ab284016)

**Storage and Stability:** Store kit at +4°C immediately upon receipt, apart from the SP Conjugate which should be stored at -20°C. Kit has a storage time of 1 year from receipt, providing components have not been reconstituted.

### Materials Supplied

Item	Quantity	Storage Condition
100X Streptavidin-Peroxidase Conjugate	80 µL	-20°C
10X Diluent M Concentrate	20 mL	4°C
20X Wash Buffer Concentrate	2 x 30 mL	4°C
50X Biotinylated Human PAK4 Antibody	1 vial	-20°C
Chromogen Substrate	7 mL	4°C
Human PAK4 Microplate	96 wells	4°C
PAK4 Standard	1 vial	4°C
Sealing Tapes	3 units	4°C
Stop Solution	11 mL	4°C

### 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 nm
- Pipettes (1-20 µL, 20-200 µL, 200-1000 µL, and multiple channel)
- Deionized or distilled reagent grade water

### Reagents Preparation

Equilibrate all reagents to room temperature (18-25°C) prior to use. The kit contains enough reagents for 96 wells.

Prepare only as much reagent as is needed on the day of the experiment.

If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved.

**Δ Note:** Concentration of the kit components are lot-specific, and the end user should always refer to the vial label.

Freshly dilute all reagents and bring all reagents to room temperature before use.

**Diluent M Concentrate (10x):** Dilute the Diluent N Concentrate 1:10 with reagent grade water to produce a 1x solution. When diluting the concentrate, make sure to rinse the bottle thoroughly to extract any precipitates left in the bottle. Mix the 1x solution gently until the crystals have completely dissolved. Store for up to 30 days at 2-8°C.

**50X Biotinylated Human PAK4 Antibody:** Spin down the antibody briefly and dilute the desired amount of the antibody 1:50 with Diluent N to produce a 1x solution. The undiluted antibody should be stored at -20°C.

**20X Wash Buffer Concentrate:** Dilute the Wash Buffer Concentrate 1:20 with reagent grade water to produce a 1x solution. When diluting the concentrate, make sure to rinse the bottle thoroughly to extract any precipitates left in the bottle. Mix the 1x solution gently until the crystals have completely dissolved.

**100X Streptavidin-Peroxidase Conjugate:** Spin down the SP Conjugate briefly and dilute the desired amount of the conjugate 1:100 with Diluent M to produce a 1x solution. The undiluted conjugate should be stored at -20°C.

### Standard Preparation

Always prepare a fresh set of standards for every use.

Prepare serially diluted standards immediately prior to use.

Any remaining standard should be stored at -20°C after reconstitution and used within 30 days. The following section describes the preparation of a standard curve for duplicate measurements (recommended).

Reconstitution of the PAK4 Standard vial to prepare a 10 ng/mL Stock Standard.

- First consult the PAK4 Standard vial to determine the mass of protein in the vial.
- Calculate the appropriate volume of 1X Diluent M to add when resuspending the PAK4 Standard vial to produce a 10 ng/mL PAK4 Stock Standard by using the following equation:
  - o  $C_s$  = Starting mass of PAK4 Standard (see vial label) (ng)
  - o  $C_f$  = The 10 ng/mL PAK4 Stock Standard final required concentration
  - o  $V_d$  = Required volume of 1X Diluent M for reconstitution (µL)
  - o Calculate total required volume 1X Diluent M for resuspension:

$$(C_s / C_f) \times 1,000 = V_d$$

**Δ Note:** This example is for demonstration purposes only. Please remember to check your standard vial for the actual amount of standard provided.

$C_s$  = 10 ng of Human PAK4 Standard in vial

$C_f$  = 10 ng/mL Human PAK4 Standard #1 final concentration

$V_d$  = Required volume of 1X Diluent M for reconstitution (10 ng / 10 ng/mL) x 1,000 = 1000 µL

- Reconstitute the Human PAK4 Standard vial by adding the appropriate calculated amount  $V_d$  of 1X Diluent M to the vial to generate the 10 ng/mL Human PAK4 Standard #1. Mix gently and thoroughly.
- Allow the reconstituted 10 ng/mL Albumin Standard #1 to sit for 10 minutes with gentle agitation prior to making subsequent dilutions
- Label five tubes #2 – 8.
- Add 120 µL of 1X Diluent N to tube #2 – 8.
- To prepare Standard #2, add 120 µL of the Standard #1 into tube #2 and mix gently.
- To prepare Standard #3, add 120 µL of the Standard #2 into tube #3 and mix gently
- Using the table below as a guide, prepare subsequent serial dilutions. 1X Diluent M serves as the zero standard (0 ng/mL).

Standard #	Volume to dilute (μL)	Volume 1X Diluent M (μL)	PAK4 (ng/ml)
1	240	-	10
2	120 μL Standard #1	120	5.0
3	120 μL Standard #2	120	2.5
4	120 μL Standard #3	120	1.25
5	120 μL Standard #4	120	0.625
6	120 μL Standard #5	120	0.313
7	120 μL Standard #6	120	0.156
8	-	120	0.000

### Sample Preparation

**Plasma:** Collect plasma using one-tenth volume of 0.1 M sodium citrate as an anticoagulant. Centrifuge samples at 3000 x g for 10 minutes, and assay. The undiluted samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles (EDTA or Heparin can also be used as anticoagulant).

**Serum:** Samples should be collected into a serum separator tube. After clot formation, centrifuge samples at 3000 x g for 10 minutes, remove serum, and assay. The undiluted samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.

**Cell Culture Supernatants:** Centrifuge cell culture media at 3000 x g for 10 minutes to remove debris. Collect supernatants and assay. Store the remaining 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).

### 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, standard solutions, and samples as instructed. Bring all reagents to room temperature before use. The assay is performed at room temperature (20-25°C).
2. Remove excess microplate strips from the plate frame and return them immediately to the foil pouch with desiccants inside. Reseal the pouch securely to minimize exposure to water vapor and store in a vacuum desiccator.
3. Add 50 μL of PAK4 Standard or sample per well. Cover wells with a sealing tape and incubate for 2 hours. Start the timer after the last addition.
4. Wash five times with 200 μL of Wash Buffer manually. Invert the plate each time and decant the contents; hit 4-5 times on absorbent material to completely remove the liquid. If using a machine, wash six times with 300 μL of Wash Buffer and then invert the plate, decanting the contents; hit 4-5 times on absorbent material to completely remove the liquid.

5. Add 50 μL of Biotinylated Human PAK4 Antibody to each well and incubate for 1 hour.
6. Wash the microplate as described above.
7. Add 50 μL of Streptavidin-Peroxidase Conjugate to each well and incubate for 30 minutes. Turn on the microplate reader and set up the program in advance.
8. Wash the microplate as described above.
9. Add 50 μL of Chromogen Substrate per well and incubate for 30 minutes or till the optimal blue color density develops. Gently tap plate to ensure thorough mixing and break the bubbles in the well with pipette tip.
10. Add 50 μL of Stop Solution to each well. The color will change from blue to yellow.
11. Read the absorbance on a microplate reader at a wavelength of 450 nm immediately. If wavelength correction is available, subtract readings at 570 nm from those at 450 nm to correct optical imperfections. Otherwise, read the plate at 450 nm only. Please note that some unstable black particles may be generated at high concentration points after stopping the reaction for about 10 minutes, which will reduce the readings.

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

[www.abcam.com/protocols/the-complete-elisa-guide](http://www.abcam.com/protocols/the-complete-elisa-guide)

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### Additional information

#### CALCULATION

1. Calculate the mean value of the duplicate or triplicate readings for each standard and sample.
2. To generate a standard curve, plot the graph using the standard concentrations on the x-axis and the corresponding mean 450 nm absorbance (OD) on the y-axis. The best-fit line can be determined by regression analysis using log-log or four-parameter logistic curve-fit.
3. Determine the unknown sample concentration from the Standard Curve and multiply the value by the dilution factor.

#### TYPICAL DATA

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

Standard Point	ng/mL	OD	Average OD
P1	10	1.787 1.808	1.757
P2	5.0	1.006 0.982	0.994
P3	2.5	0.608 0.575	0.591
P4	1.25	0.388 0.375	0.382
P5	0.625	0.259 0.256	0.258
P6	0.313	0.200 0.202	0.201
P7	0.156	0.158 0.162	0.160
P8	0.000	0.123 0.129	0.126
Sample: Normal, Sodium Citrate Plasma (1x)		0.278	0.278

#### PERFORMANCE CHARACTERISTICS

- The minimum detectable dose of PAK4 as calculated by 2SD from the mean of a zero standard was established to be 0.1 ng/ml.
- Intra-assay precision was determined by testing replicates of three plasma samples in one assay.
- Inter-assay precision was determined by testing three plasma samples in twenty assays.

	Intra-Assay Precision	Inter-Assay Precision
CV (%)	4.2	7.4

#### RECOVERY

Standard Added Value	0.3 – 5 ng/mL
Recovery %	91 – 108%
Average Recovery %	96%

#### LINEARITY

Average Percentage of Expected Value (%)		
Sample Dilution	Plasma	Serum
No Dilution	94%	93%
1:2	101%	100%
1:4	104%	102%

#### CROSS REACTIVITY

Species	Cross-Reactivity (%)
Canine	None
Bovine	None
Monkey	90
Mouse	None
Rat	90
Swine	90
Rabbit	None

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