antibody and ELISA experts

PicoKine™ Quick ELISA Kit

Catalog number: FEK0755

For the quantitation of **Human EPCAM** concentrations in cell culture supernatants, cell lysates, serum and plasma (heparin, EDTA).

This package insert must be read in its entirety before using this product.
For research use only. Not for use in diagnostic procedures.

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Human EPCAM/Trop1 PicoKine® Quick ELISA Kit

Catalog Number:FEK0755

Assay Principle

The Boster Picokine™ Human EPCAM Pre-Coated ELISA (Enzyme-Linked Immunosorbent Assay) kit is a solid phase immunoassay specially designed to measure Human EPCAM with a 96-well strip plate that is pre-coated with antibody specific for EPCAM. The detection antibody is a biotinylated antibody specific for EPCAM. The capture antibody is monoclonal antibody from mouse and the detection antibody is polyclonal antibody from goat. The kit includes Human EPCAM protein as standards.

To measure Human EPCAM, add standards and samples to the wells, then add the biotinylated detection antibody. Wash the wells with PBS or TBS buffer, and add Avidin-Biotin-Peroxidase Complex (ABC-HRP). Wash away the unbounded ABC-HRP with PBS or TBS buffer and add TMB. TMB is substrate to HRP and will be catalyzed to produce a blue color product, which changes into yellow after adding acidic stop solution. The density of the yellow product is linearly propotional to Human EPCAM in the sample. Read the density of the yellow product in each well using a plate reader, and benchmark the sample wells' readings against the standard curve to determine the concentration of Human EPCAM in the sample. For more information on assay principle, protocols, and troubleshooting tips, see Boster's ELISA Resource Center at https://www.bosterbio.com/elisa-technical-resource-center.

Overview

Product Name	Human EPCAM/Trop1 PicoKine® Quick ELISA Kit
Reactive Species	Human
Size	96 wells/kit, with removable strips.
Description	Human EPCAM/Trop1 PicoKine™ Quick ELISA Kit (90 minutes, 96 Tests). Quantitate Human EPCAM in cell culture supernatants, cell lysates, serum and plasma (heparin, EDTA). Sensitivity: 10pg/ml.
Sensitivity	<10 pg/ml *The sensitivity or the minimum detectable dose (MDD) is the lower limit of target protein that can be detected by the kit. It is determined by adding two standard deviations to the mean O.D. value of twenty (20) blank wells and calculating the corresponding concentration.
Detection Range	15.6 pg/ml - 1,000 pg/ml
Storage Instructions	Store at 4°C for 6 months, at -20°C for 12 months. Avoid multiple freeze-thaw cycles (Ships with gel ice, can store for up to 3 days in room temperature. Freeze upon receiving.)
Uniprot ID	P16422

Technical Details

Capture/Detection Antibodies	The capture antibody is monoclonal antibody from mouse and the detection antibody is polyclonal antibody from goat.

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Specificity	Natural and recombinant Human EPCAM
Standard	Expression system for standard: NS0; Immunogen sequence: Q24 - K265
Cross Reactivity	There is no detectable cross-reactivity with other relevant proteins.

Kit Components/Materials Provided

Description	Quantity	Volume
Anti-Human EPCAM Pre-coated 96-well strip microplate	1	12 strips of 8 wells
Human EPCAM Standard	2	10 ng/tube
Human EPCAM Biotinylated antibody (50x)	1	100 µl
Avidin-Biotin-Peroxidase Complex (30x)	1	400 µl
Sample Diluent	1	30 ml
Antibody Diluent	1	12 ml
Avidin-Biotin-Peroxidase Diluent	1	12 ml
Wash Buffer (25x)	1	20 ml
Color Developing Reagent (TMB)	1	10 ml
Stop Solution	1	10 ml
Plate Sealers	4	Piece

Required Materials That Are Not Supplied

Microplate Reader capable of reading absorbance at 450nm.
Automated plate washer (optional)
Pipettes and pipette tips capable of precisely dispensing 0.5 µl through 1 ml volumes of aqueous solutions.
Multichannel pipettes are recommended for large amount of samples.
Deionized or distilled water.
500ml graduated cylinders.
Test tubes for dilution.

Notice Before Application

Please read the following instructions before starting the experiment.
1. To inspect the validity of experiment operation and the appropriateness of sample dilution proportion, pilot experiment using

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standards and a small number of samples is recommended.
2. Before using the Kit, spin tubes and bring down all components to the bottom of tubes.
3. Don't let 96-well plate dry, for dry plate will inactivate active components on plate.
4. Don't reuse tips and tubes to avoid cross contamination.
5. Avoid using the reagents from different batches together.

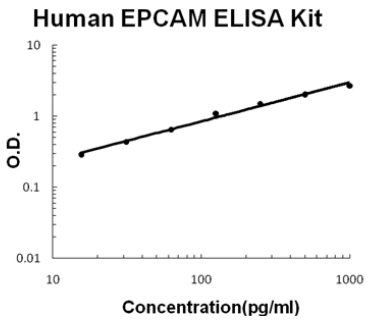
Human EPCAM/Trop1 PicoKine® Quick ELISA Kit (FEK0755) Standard Curve Example

Highest O.D. value might be higher or lower than in the example. The experiment result is statistically significant if the highest O.D. value is no less than 1.0.

Concentration (pg/ml)	0	15.6	31.2	62.5	125	250	500	1000
O.D.	0.024	0.288	0.433	0.647	1.088	1.501	2.024	2.689

Human EPCAM PicoKine ELISA Kit standard curve

A standard curve is provided for demonstration only. A standard curve should be generated for each set of samples assayed.



Intra/Inter Assay Variability

Boster spend great efforts in documenting lot to lot variability and make sure our assay kits produce robust data that are reproducible.

Intra-Assay Precision (Precision within an assay): Three samples of known concentration were tested on one plate to assess intra-assay precision.

Inter-Assay Precision (Precision accross assays): Three samples of known concentration were tested in separate assays to assess inter-assay precision.

	Intra-Assay Precision			Inter-Assay Precision		
Sample	1	2	3	1	2	3
n	16	16	16	24	24	24
Mean (pg/ml)	38	143	444	35	150	444
Standard deviation	1.63	6	33.74	1.82	7.5	37.29
CV (%)	4.3%	4.2%	7.6%	5.2%	5%	8.4%

Reproducibility

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We ensure reproducibility by testing three samples with differing concentrations of Bdnf in ELISA kits from four different production batches/lots.

Lots	Lot 1 (pg/ml)	Lot 2 (pg/ml)	Lot 3 (pg/ml)	Lot 4 (pg/ml)	Mean (pg/ml)	Standard Deviation	CV (%)
Sample 1	38	35	32	35	35	2.12	6%
Sample 2	143	161	140	157	150	8.92	5.9%
Sample 3	444	418	452	413	431	16.58	3.8%

*number of samples for each test n=16.

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Preparation Before The Experiment

Item	Preparation
All reagents	Bring all reagents to 37°C prior to use. Also the TMB incubation time estimate (20-25min) is based on 37°C.
Wash buffer	Prepare 500 ml of Working Wash Buffer by diluting the supplied 20 ml of Wash Buffer (25 x) with 480 ml of deionized or distilled water. If crystals have formed in the concentrate, warm to room temperature and mix it gently until crystals have completely dissolved.
Biotinylated Anti-Human EPCAM antibody	It is recommended to prepare this reagent immediately prior to use by diluting the Human EPCAM Biotinylated antibody (50x) 1:50 with Antibody Diluent. Prepare 50 µl by adding 1 µl of Biotinylated antibody (50x) to 49 µl of Antibody Diluent. Mix gently and thoroughly and use within 2 hours of generation.
Avidin-Biotin-Peroxidase Complex	It is recommended to prepare this reagent immediately prior to use by diluting the Avidin-Biotin-Peroxidase Complex (30x) 1:30 with Avidin-Biotin-Peroxidase Diluent. Prepare 400 µl by adding 10 µl of Avidin-Biotin-Peroxidase Complex (30x) to 390 µl of Avidin-Biotin-Peroxidase Diluent. Mix gently and thoroughly and use within 2 hours of generation.
Human EPCAM Standard	It is recommended that the standards be prepared no more than 2 hours prior to performing the experiment. Use one 10 ng of lyophilized Human EPCAM standard for each experiment. Gently spin the vial prior to use. Reconstitute the standard to a stock concentration of 10 ng/ml using 1ml of sample diluent. Allow the standard to sit for a minimum of 10 minutes with gentle agitation prior to making dilutions.
Microplate	The included microplate is coated with capture antibodies and ready-to-use. It does not require additional washing or blocking. The unused well strips should be sealed and stored in the original packaging.

Dilution of Human EPCAM Standard

1. Number tubes 1-8. Final Concentrations to be Tube # 1: 1,000.00 pg/ml, # 2: 500.00 pg/ml, # 3: 250.00 pg/ml, # 4: 125.00 pg/ml,
   # 5: 62.50 pg/ml, # 6: 31.25 pg/ml, # 7: 15.63 pg/ml, # 8: Sample Diluent serves as the zero standard (0 pg/ml).
2. To generate standard #1, add 100 µl of the reconstituted standard stock solution of 10 ng/ml and 900 µl of sample diluent to tube #1 for a
   final volume of 1000 µl. Mix thoroughly.
3. Add 300 µl of sample diluent to tubes # 2-7.
4. To generate standard # 2, add 300 µl of standard # 1 from tube # 1 to tube # 2 for a final volume of 600 µl. Mix thoroughly.
5. To generate standard # 3, add 300 µl of standard # 2 from tube # 2 to tube # 3 for a final volume of 600 µl. Mix thoroughly.
6. Continue the serial dilution for tube # 4-7.

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Sample Preparation and Storage

These sample collection instructions and storage conditions are intended as a general guideline and the sample stability has not been evaluated.

Sample Type	Procedure
Cell culture supernatants	Clear sample of particulates by centrifugation, assay immediately, or store samples at -20°C.
Serum	Use a serum separator tube (SST) and allow serum to clot at room temperature for about four hours. Then, centrifuge for 15 min at approximately 1,000 x g. assay immediately or store samples at -20°C.
Plasma	Collect plasma using heparin or EDTA as an anticoagulant. Centrifuge for 15 min at approximately 1,000 x g. Assay immediately or store samples at -20°C. *Note: it is important to not use anticoagulants other than the ones described above to treat plasma, for other anticoagulants could block the antibody binding site.
Cell lysates	Lyse the cells, make sure there are no visible cell sediments. Centrifuge cell lysates at approximately 10,000 x g for 5 min. Collect the supernatant.

Sample Dilution

The target protein concentration should be estimated and appropriate sample dilutions should be selected such that the final protein concentration lies near the middle of the linear dynamic range of the assay.

It is recommended to prepare 150 µl of sample for each replicate to be assayed. The samples should be diluted with sample diluent and mixed gently.

Assay protocol

It is recommended that all reagents and materials be equilibrated to 37°C/room temperature prior to the experiment (see Preparation Before The Experiment if you have missed this information).

1. Prepare all reagents and working standards as directed previously.
2. Remove excess microplate strips from the plate frame and seal and store them in the original packaging.
3. Add 50 µl of the standard, samples, or control per well. And add 50µl of the prepared 1x Biotinylated Anti-Human EPCAM antibody per well. Add 50 µl of the sample diluent buffer and 50µl of the prepared 1x Biotinylated Anti-Human EPCAM antibody into the control well (Zero well). At least two replicates of each standard, sample, or control is recommended.
4. Cover with the plate sealer provided and incubate for 60 minutes at RT.
5. Wash the plate 3 times with the 1x wash buffer.
   a. Discard the liquid in the wells into an appropriate waste receptacle. Then, invert the plate on the benchtop onto a paper towel and tap the plate to gently blot any remaining liquid. It is recommended that the wells are not allowed to completely dry at any time.
   b. Add 300 µl of the 1x wash buffer to each assay well. (For cleaner background incubate for 60 seconds between each wash).
   c. Repeat steps a-b 2 additional times.
6. Add 100 µl of the prepared 1x Avidin-Biotin-Peroxidase Complex into each well. Cover with plate sealer provided and incubate for 15 minutes at RT.
7. Wash the plate 5 times with the 1x wash buffer.
   a. Discard the liquid in the wells into an appropriate waste receptacle. Then, invert the plate on the benchtop onto a paper towel and tap the plate

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to gently blot any remaining liquid. It is recommended that the wells are not allowed to completely dry at any time.
b. Add 300 µl of the 1x wash buffer to each assay well. (For cleaner background incubate for 60 seconds between each wash).
c. Repeat steps a-b 4 additional times.
8. Add 90 µl of Color Developing Reagent to each well and incubate in the dark for 30 minutes at RT (or 25-30 minutes at 37°C). (The optimal incubation time must be empirically determined. A guideline to look for is blue shading the top four standard wells, while the remaining standards remain clear.)
9. Add 100 µl of Stop Solution to each well. The color should immediately change to yellow.
10. Within 30 minutes of stopping the reaction, the O.D. absorbance should be read with a microplate reader at 450nm.

Data Analysis

Average the duplicate readings for each standard, sample, and control. Subtract the average zero standard O.D. reading.

It is recommended that a standard curve be created using computer software to generate a four parameter logistic (4-PL) curve-fit. A free program capable of generating a four parameter logistic (4-PL) curve-fit can be found online at: www.myassays.com/four-parameter-logistic-curve.assay.

Alternatively, plot the mean absorbance for each standard against the concentration. The measured concentration in the sample can be interpolated by using linear regression of each average relative OD against the standard curve generated using curve fitting software. This will generate an adequate but less precise fit of the data.

For diluted samples, the concentration reading from the standard curve must be multiplied by the dilution factor.

Background on EPCAM

Epithelial cell adhesion molecule (EpCAM) is a transmembrane glycoprotein mediating Ca2+-independent homotypic cell-cell adhesion in epithelia. Also it is involved in cell signaling, migration, proliferation, and differentiation. Additionally, EpCAM has oncogenic potential via its capacity to upregulate c-myc, e-fabp, and cyclins A & E. Since it is expressed exclusively in epithelia and epithelial-derived neoplasms, EpCAM can be used as diagnostic marker for various cancers. It appears to play a role in tumorigenesis and metastasis of carcinomas, so it can also act as a potential prognostic marker and as a potential target for immunotherapeutic strategies.

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