antibody and ELISA experts

PicoKine® Quick ELISA Kit

Catalog number: FEK0838

For the quantitation of **Human DIABLO** 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 SMAC PicoKine® Quick ELISA Kit

Catalog Number: FEK0838

Assay Principle

The Boster Quick Picokine® Human DIABLO Pre-Coated ELISA (Enzyme-Linked Immunosorbent Assay) kit is a solid phase immunoassay specially designed to measure Human DIABLO in cell culture supernatants, cell lysates, serum and plasma (heparin, EDTA).. It uses our proprietary Quick ELISA technology. Quick ELISA technology employs capture antibodies conjugated to an affinity tag that is recognized by the antibody used to coat our Quick ELISA plates. This approach to sandwich ELISA allows the formation of the antibody-analyte sandwich complex in a single step, significantly reducing assay time. The kit includes Human DIABLO protein as standards.

To measure Human DIABLO, add standards and samples to the wells, then add antibody cocktail. Wash the wells with PBS or TBS buffer, and add TMB. TMB is an HRP substrate and will be catalyzed to produce a blue color product, which changes into yellow after adding the acidic stop solution. The absorbance of the yellow product is linearly proportional to Human DIABLO in the sample. Read the absorbance 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 DIABLO 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.

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Overview

Product Name	Human SMAC PicoKine® Quick ELISA Kit
Reactive Species	Human
Size	96 wells/kit, with removable strips.
Description	Human Diablo/SMAC PicoKine® Quick ELISA Kit (90 minues, 96 Tests). Quantitate Human DIABLO in cell culture supernatants, cell lysates, serum and plasma (heparin, EDTA). Sensitivity: 10pg/ml. The brand Picokine indicates this is a premium quality ELISA kit. Each Picokine kit delivers precise quantification, high sensitivity, and excellent reproducibility. Only our most reliable and effective kits qualify as Picokine, guaranteeing top-tier results for your assays.
Sensitivity	<10 pg/ml *The sensitivity or the minimum detectable dose (MDD) is the lower limit of the 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	31.2 pg/ml - 2,000 pg/ml
Storage Instructions	Store at 4°C for 6 months. (Ships with gel ice, can store for up to 3 days in room temperature. Refrigerate upon receipt.)

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Technical Details

Specificity	Natural and recombinant Human DIABLO
Standard Protein	Expression system for standard: E.coli; Immunogen sequence: A56-D239
Cross Reactivity	There is no detectable cross-reactivity with other relevant proteins.

Notice Before Application

Please read the following instructions before starting the experiment.
1. Read this manual in its entirety in order to minimize the chance of error.
2. Confirm that you have the appropriate non-supplied equipment available.
3. Confirm that the species, target antigen, and sensitivity of this kit are appropriate for your intended application.
4. Confirm that your samples have been prepared appropriately based upon recommendations (see Sample Preparation) and that you have sufficient sample volume for use in the assay.
5. When first using a kit, appropriate validation steps should be taken before using valuable samples. Confirm that the kit adequately detects the target antigen in your intended sample type(s) by running control samples.
6. If the concentration of target antigen within your samples is unknown, a preliminary experiment should be run using a control sample to determine the optimal sample dilution (see Sample Preparation).
7. To inspect the validity of experiment operation and the appropriateness of sample dilution proportion, a pilot experiment using standards and a small number of samples is recommended.
8. Before using the kit, spin tubes to bring down all components to the bottom of the tubes.
9. Don't let the 96-well plate dry out since this will inactivate active components on the plate.
10. Don't reuse tips and tubes to avoid cross-contamination.
11. Avoid using the reagents from different batches together.
12. The kit should not be used beyond the expiration date on the kit label. Any variation in diluent, operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding. Variations in sample collection, processing, and storage may cause sample value differences.

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Kit Components/Materials Provided

Catalog number	Description	Quantity	Volume
FEK0838-CAP	Anti-tag Pre-coated 96-well Strip Microplate	1	12 strips of 8 wells
EK0838-ST	Human DIABLO Standard	2	10 ng/tube
FEK0838-A	Human DIABLO Antibody A	1	3 ml
FEK0838-B	Human DIABLO Antibody B	1	3 ml
AR1106-1	Sample Diluent	1	15 ml
AR1106-7	TBS-T Wash Buffer (25x)	1	12 ml
AR1104	Color Developing Reagent (TMB)	1	10 ml
AR1105	Stop Solution	1	10 ml
PLA-SEA	Adhesive Plate Sealers	2	Piece

Required Materials That Are Not Supplied

Microplate reader capable of reading absorbance at 450 nm.
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 a large numbers of samples.
Deionized or distilled water.
500 ml graduated cylinders.
Test tubes for dilution.
Horizontal orbital microplate shaker capable of maintaining a speed of 500 rpm, amplitude 3 mm.

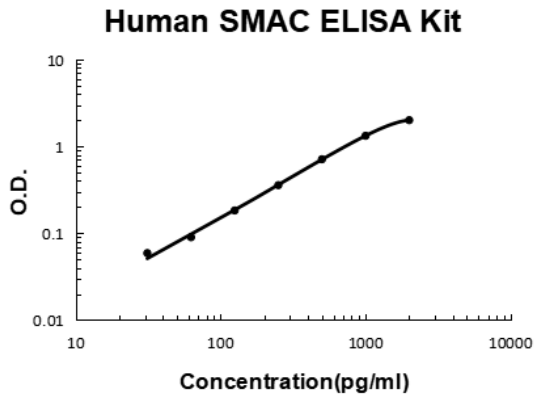
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Human SMAC PicoKine® Quick ELISA Kit (FEK0838) Standard Curve Example

The 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	31.2	62.5	125	250	500	1000	2000
O.D.	0.013	0.072	0.103	0.196	0.371	0.725	1.340	2.021

Human SMAC 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 ensuring 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 across 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)	54	221	865	53	229	932
Standard deviation	4.05	17.01	38.83	4.61	20.15	48.46
CV (%)	7.5%	7.7%	4.2%	8.7%	8.8%	5.2%

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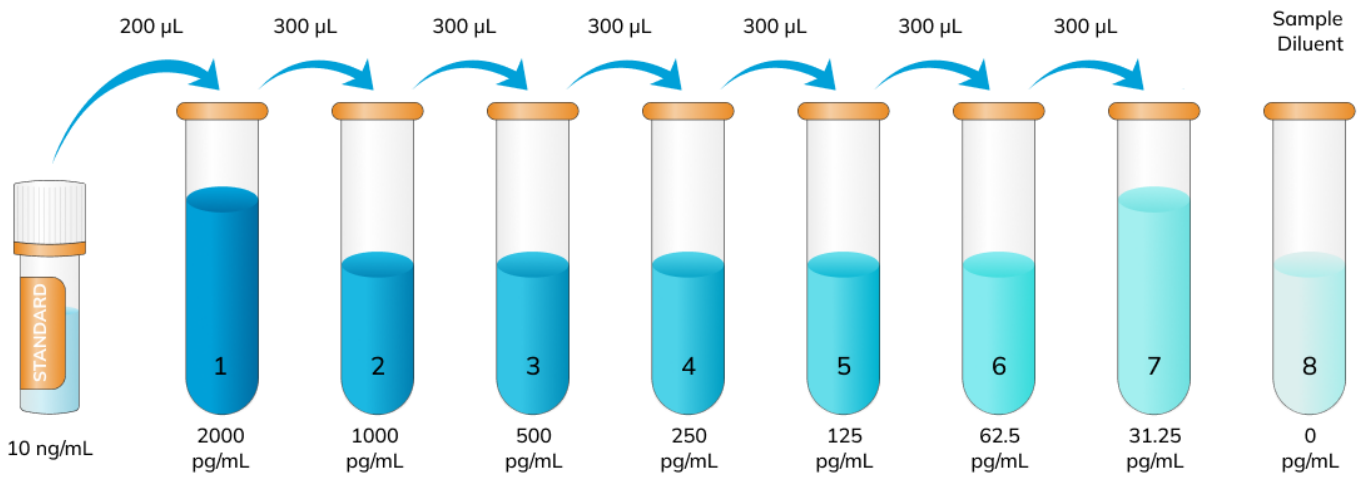
Preparation Before The Experiment

Item	Preparation
All reagents	Bring all reagents to room temperature (18-25°C) prior to use. Please DO NOT equilibrate unused plate well strips to room temperature. They should be sealed and stored in the original packaging. We recommend doing it at 37°C for best consistency with our QC results. Also, the TMB incubation time estimate (15-25 min) is based on incubation at 37°C. It is recommended that all reagents be prepared no more than 1 hour prior to performing the experiment.
TBS-T Wash Buffer (25x)	Add 12 ml of Wash Buffer into 288 ml of deionized water.
Human DIABLO Standard	Use one 10 ng of lyophilized Human DIABLO 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.
Human DIABLO Antibody Cocktail	Mix Antibody A and B at dilution ratio of 1:1.
Microplate	The included microplate is coated with capture antibodies and is ready-to-use. It does not require additional washing or blocking. The unused well strips should be sealed and stored in the original packaging.

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Dilution of Human DIABLO Standard

1. Number tubes 1-8. Final Concentrations to be Tube # 1: 2,000.00 pg/ml, # 2: 1,000.00 pg/ml, # 3: 500.00 pg/ml, # 4: 250.00 pg/ml,
   # 5: 125.00 pg/ml, # 6: 62.50 pg/ml, # 7: 31.25 pg/ml, # 8: Sample Diluent serves as the zero standard (0 pg/ml).
2. To generate standard #1, add 200 µl of the reconstituted standard stock solution of 10 ng/ml and 800 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 Collection Notes

1. Boster recommends that samples are used immediately upon preparation.
2. Avoid repeated freeze/thaw cycles for all samples.
3. In the event that a sample type not listed above is intended to be used with the kit, it is recommended that the customer conduct validation experiments in order to be confident in the results.
4. Due to chemical interference, the use of tissue or cell extraction samples prepared by chemical lysis buffers may result in inaccurate results.
5. Due to factors including cell viability, cell number, or sampling time, samples from cell culture supernatant may not be detected by the kit.
6. Samples should be brought to room temperature (18-25°C) before performing the assay without the use of extra heating.
7. Sample concentrations should be predicted before being used in the assay. If the sample concentration is not within the range of the standard curve, users must determine the optimal sample dilutions for their particular experiments.
8. Boster is responsible for the quality and performance of the kit components but is NOT responsible for the performance of customer supplied samples used with the kit.

Sample Dilution

The user needs to estimate the concentration of the target protein in the sample and use an appropriate dilution factor so that the diluted target protein concentration falls in the range of O.D. values of the standard curve. Dilute the sample using provided diluent buffer. Pilot tests using a dilution series of each sample type is necessary. The sample must be mixed thoroughly with Sample Diluent.

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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. Add 50µl of Sample Diluent into the Zero well. At least two replicates of each standard, sample, or control is recommended.
4. And add 50µl of Human DIABLO Antibody Cocktail per well.
5. Cover with the plate sealer provided and incubate for 60 minutes at room temperature on the shaker.
6. Wash the plate 4 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 90 seconds between each wash).
 c. Repeat steps a-b 2 additional times.
 Discard the wash buffer 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.
7. Add 90 µl of Color Developing Reagent to each well and incubate in the dark for 15 minutes at RT. (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.)
8. Add 100 µl of Stop Solution to each well. The color should immediately change to yellow.
9. 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 O.D. 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.

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Background on DIABLO

Diablo homolog, mitochondrial, also known as second mitochondria-derived activator of caspases (SMAC), is a protein that in humans is encoded by the DIABLO gene (IAP binding protein with low pI). It is mapped to 12q24.31. DIABLO can bind mammalian IAP homolog A (MIHA, or API3) and can also interact with MIHB, MIHC, and OpIAP, the baculoviral IAP. This gene encodes an inhibitor of apoptosis protein (IAP)-binding protein. The encoded mitochondrial protein enters the cytosol when cells undergo apoptosis, and allows activation of caspases by binding to inhibitor of apoptosis proteins. Over expression of the encoded protein sensitizes tumor cells to apoptosis. A mutation in this gene is associated with young-adult onset of nonsyndromic deafness-64.

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