ICOS (Human) ELISA Kit

rev 03/17

(Catalog # K4176-100, 100 assays, Store at -20°C)

I. Introduction:

ICOS enhances all basic T-cell responses to a foreign antigen, namely proliferation, secretion of lymphokines, up-regulation of molecules that mediate cell-cell interaction, and effective help for antibody secretion by B-cells. Essential both for efficient interaction between T and B-cells and for normal antibody responses to T-cell dependent antigens. Does not up-regulate the production of interleukin-2, but superinduces the synthesis of interleukin-10. Prevents the apoptosis of pre-activated T-cells. Plays a critical role in CD40-mediated class switching of immunoglobin isotypes. BioVision's ICOS ELISA kit is a sandwich ELISA assay for the quantitative measurement of human ICOS in serum, plasma and cell culture supernatants. The density of color is proportional to the amount of ICOS captured from the samples.

II. Application:

Quantitative protein detection, establishing normal range, validation of antibody array results.
Detection Range: 1.23 - 300 ng/ml
Sensitivity: < 1.2 ng/ml

III. Sample Type:

Human serum, plasma, and cell culture supernatants

IV. Kit Contents:

Components	K4176-100	Part No.	Storage / Stability After Preparation
ICOS Microplate (Item A): 96 wells	12 strips x 8 wells	K4176-100-1	1 month at 4°C
Wash Buffer Concentrate (20X) (Item B)	25 ml	K4176-100-2	1 month at 4°C
Standard Protein (Item C)	2 vials	K4176-100-3	1 week at -80°C
Detection Antibody ICOS (Item F)	2 vials	K4176-100-4	5 days at 4°C
HRP-Streptavidin Concentrate (400X) (Item G)	200 µl	K4176-100-5	Do not store and reuse
TMB One-Step Substrate Reagent (Item H)	12 ml	K4176-100-6	--
Stop Solution (Item I)	8 ml	K4176-100-7	--
Assay Diluent D (Item K)	15 ml	K4176-100-8	1 month at 4°C
Assay Diluent B (5X) (Item E)	15 ml	K4176-100-9	1 month at 4°C

V. User Supplied Reagents and Equipment:

- Microplate reader capable of measuring absorbance at 450 nm
- Precision pipettes to deliver 2 µl to 1 ml volumes
- Adjustable 1-25 ml pipettes for reagent preparation
- 100 ml and 1 liter graduated cylinders.
- Clean eppendorf tubes for preparing standards or sample dilutions

VI. Storage and Handling:

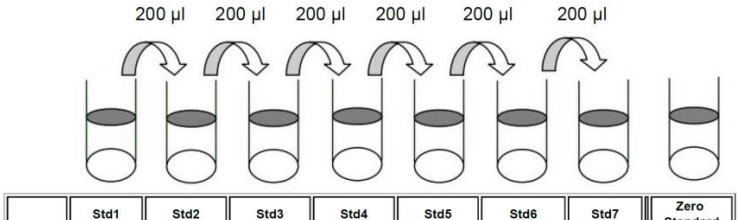
The entire kit may be stored at -20°C for up to 1 year from the date of shipment. It is recommended to store at -80°C for extended storage. Avoid repeated freeze-thaw cycles.

VII. Reagent Preparation:

Note: Bring all reagents and samples to room temperature before use.

Reagent Dilution:

1. **Assay Diluent D** (Item K) and **Assay Diluent B** (Item E) should be diluted 5-fold with deionized or distilled water before use.
2. If the **Wash Concentrate** (20X) (Item B) contains visible crystals, warm to room temperature and mix gently until dissolved. Dilute 20 ml of Wash Buffer Concentrate into deionized or distilled water to yield 400 ml of 1X Wash Buffer.
3. Briefly spin the **Detection Antibody** (Item F) before use. Add 100 µl of 1X Assay Diluent B (Item E) into the vial to prepare a detection antibody concentrate. Pipette up and down to mix gently (the concentrate can be stored at 4ºC for 5 days). The detection antibody concentrate should be diluted 80- fold with 1X Assay Diluent B (Item E).
4. Briefly spin the **HRP-Streptavidin Concentrate** (Item G) and pipette up and down to mix gently before use, as precipitates may form during storage. HRP-Streptavidin concentrate should be diluted 400-fold with 1X Assay



	Std1	Std2	Std3	Std4	Std5	Std6	Std7	Zero Standard
Diluent volume	Item C + 400 µl	400 µl	400 µl	400 µl	400 µl	400 µl	400 µl	400 µl
Conc.	300 ng/ml	120 ng/ml	48 ng/ml	19.2 ng/ml	7.68 ng/ml	3.072 ng/ml	1.229 ng/ml	0 ng/ml

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Diluent B (Item E).

5. **Sample Dilution**: Sample dilution: 1X Assay Diluent D (Item K) should be used for dilution of serum, plasma, and cell culture supernatant samples. The suggested dilution for normal serum/plasma is 4 fold. However, Levels of ICOS may vary between different samples. Optimal dilution factors for each sample must be determined by the investigator.
6. **Standard Preparation**: Preparation of standard: Briefly spin a vial of Item C. Add 400 µl 1X Assay Diluent D (Item K, Assay Diluent D should be diluted 5-fold with deionized or distilled water before use) into Item C vial to prepare a 300 ng/ml standard solution. Dissolve the powder thoroughly by a gentle mix. Pipette 400 µl 1x Assay Diluent D into each tube. Use 300 ng/ml standard solution to produce a dilution series (shown below). Mix each tube thoroughly before the next transfer. 1x Assay Diluent D serves as the zero standard (0 ng/ml).

VIII. Assay Protocol:

Note: Bring all reagents and samples to room temperature before use.
It is recommended that all standards and samples be run at least in duplicate.

1. Label removable 8-well strips as appropriate for your experiment.
2. Add 100 µl of each **standard** and **samples** into appropriate wells. Cover well and incubate for 2.5 hours at room temperature or over-night at 4ºC with gentle shaking.
3. Discard the solution and wash 4 times with **1X Wash Solution**. Wash by filling each well with Wash Buffer (300 µl) using a multi-channel Pipette or autowasher. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
4. Add 100 µl of prepared **1X Detection Antibody** to each well. Incubate for 1 hour at room temperature with gentle shaking. Discard the solution. Repeat the wash as in step 3.
5. Add 100 µl of prepared **Streptavidin Solution** to each well. Incubate for 45 minutes at room temperature with gentle shaking. Discard the solution. Repeat the wash as in step 3.
6. Add 100 µl of **TMB One-Step Substrate Reagent** (Item H) to each well. Incubate for 30 minutes at room temperature in the dark with gentle shaking.
7. Add 50 µl of **Stop Solution** (Item I) to each well. Read result at 450 nm immediately.

IX. CALCULATION:

Calculate the mean absorbance for each set of duplicate standards, controls and samples, and subtract the average zero standard optical density. Plot the standard curve on log-log graph paper or using Sigma plot software, with standard concentration on the x-axis and absorbance on the y-axis. Draw the best-fit straight line through the standard points.

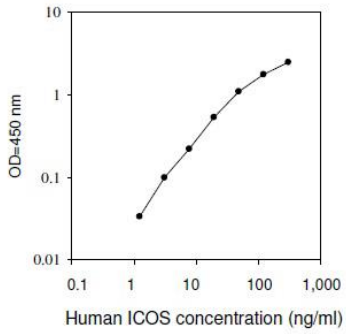

Figure 1: Standard Curve: These standard curves are for demonstration only. A standard curve must be run with each assay.

Sample Type	Average % Recovery	Range (%)
Serum	84.10	76-94
Plasma	81.74	77-86
Cell culture media	102.0	97-106

Table 1: Spiking and Recovery. Recovery was determined by spiking various levels of Human ICOS into the sample types listed above.

Sample Type		Serum	Plasma	Cell Culture Media
1:2	Average % of Expected	109.0	101.4	98.86
	Range (%)	97-121	91-111	91-107
1:4	Average % of Expected	107.0	106.7	99.73
	Range (%)	98-116	96-123	89-105

Table 2: Linearity

X. RELATED PRODUCTS:

- Human CellExp™ ICOS / CD278, Human recombinant (Cat. No. 9238-10, -100)
- Human CellExp™ ICOSLG /B7-H2 /CD275, human recombinant (Cat. No. 7426-20, -100)
- PD-1 (human) ELISA Kit (Cat. No. K4153-100)
- PD-L1 (human) ELISA Kit (Cat. No. K4155-100)
- CTLA4 /CD152 (Human) ELISA Kit (Cat. No. K4157-100)
- OX40/CD134 (Human) ELISA Kit (Cat. No. K4173-100)

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