HUMAN CD48 QUANTIFICATION KIT
INSTRUCTION MANUAL

APPLICATION

The human CD48 Quantification kit provides a rapid and easy method for the quantitative determination of human CD48 in cell culture supernatant, serum and plasma. The kit includes ready-to-use reagents necessary to analyse up to 88 samples in 3 hours.

PRINCIPLE OF THE ASSAY

The human CD48 test is based on the quantitative sandwich enzyme immunoassay technique. Microtiter wells are pre-coated with human CD48-specific polyclonal capture antibodies. Samples and standards are pipetted into microwells and human CD48 molecules present in the sample are bound by the capture antibodies. After incubation, unbound material is removed by washing the wells. Then, horseradish peroxidase (HRP) conjugated human CD48-specific polyclonal detection antibodies bind to different epitopes of human CD48 molecules. After washing, the ready to use HRP substrate (TMB) is added to wells. The intensity of the colour produced is directly proportional to the amount of human CD48 in the sample. Colour development is then stopped by the addition of stop solution. Absorbance is measured at 450 nm.

SENSITIVITY

The detection range is from 25 pg/mL to 1600 pg/mL.
The detection limit is 2 pg/mL to 8 pg/mL, defined by the minimum human CD48 concentration deviating by 2 standard deviations (2SD) from that of the standard A. The test was performed by using 16 replicate determinations of standard A (blank) and standard B.

STORAGE CONDITIONS

The kit should be stored at +2...+6°C. Unopened, the kit will remain stable until the expiry date printed on the kit label. The expiry date of each unopened component is printed on the label of the individual component. After opening, the components should be used within 8 weeks (microwell plate desiccation recommended).

KIT CONTENTS

- Pre-coated microwell plates: 96 microwells coated with anti-human CD48 rabbit polyclonal antibodies.
- Human CD48 sample diluent, 25 mL, pink solution (PBS pH7.4, BPLA, detergent and preservative)
- Human CD48 standards A-H, 1 mL, pink solution (0-25-50-100-200-400-800-1600 pg/mL)
- Human CD48 enzyme conjugate stock, 30 µL, (HRP-conjugated rabbit polyclonal antibodies in a stabilizing solution)
- Human CD48 enzyme conjugate stock diluent, 12 mL, blue solution (PBS pH7.4, BPLA, detergent and preservative)
- Wash concentrate, 50 mL
  (PBS pH 7.4 and detergent)
- Substrate solution (TMB), 12 mL
- Stop solution (0.5 M H_2SO_4), 12 mL

MATERIALS AND EQUIPMENT REQUIRED

- Pipettes and tips (1-5000 µl)
- Microplate reader (450 nm)
- Lid or sealing tape for microwell plate
- Microwell plate shaker

ASSAY PROCEDURE

Allow all reagents to reach room temperature (RT) (20°-22° C) before use (30 minutes), except the enzyme conjugate stock. Take the required number of microplate strips and place the remaining strips back into the vacuum bag. Close the bag tightly.

STEP 1	Dilute 50 mL of wash concentrate with 450 mL of distilled water to prepare washing solution.
STEP 2	Perform dilutions of each sample in sample diluent (pink).
STEP 3	Add 100 µL of samples and standards (pink) into appropriate wells in duplicate.
STEP 4	Incubate the covered microplate for 60 min at RT on a microwell plate shaker (300 rpm).
STEP 5	Prepare enzyme conjugate working solution by diluting enzyme conjugate stock in enzyme conjugate stock diluent (blue) (1:200 to 1:800) (the precise dilution factor comes with the kit).
STEP 6	Discard the solution and wash the wells 4 times with 300 µL of washing solution.
STEP 7	Add 100 µL of enzyme conjugate working solution into each well.
STEP 8	Incubate the covered microplate for 90 min at RT on a microwell plate shaker (300 rpm).
STEP 9	Discard the solution and wash the wells 4 times with 300 µL of washing solution.
STEP 10	Add 100 µL of substrate solution into each well.
STEP 11	Incubate the covered microplate for 10 - 25 minutes (the precise incubation time comes with the kit) at RT on a microwell plate shaker (300 rpm).
STEP 12	Stop the reaction by adding 50 µl of STOP solution into each well in the same order and time as for TMB distribution.
STEP 13	Read the absorbance at 450 nm immediately.

PREPARATION OF SAMPLES

Dilute the samples in sample diluent.

PREPARATION OF ENZYME CONJUGATE WORKING SOLUTION

The enzyme conjugate working solution should be prepared before use. Prepare the enzyme conjugate working solution by diluting enzyme conjugate stock in

enzyme conjugate stock diluent (1:200 to 1:800) (the precise dilution factor comes with the kit). Discard any remaining working solution after use.

MICROWELL PLATE WASH

It is recommended that the microwell plate wells be washed by hand (e.g. using a multi-channel pipette) during the washing steps, as a plate washer may cause poor assay precision.

CALCULATION OF RESULTS

Standard curve: Calculate the mean absorbance for each standard. Subtract the blank value (standard A) from the mean absorbances. Plot the value (absorbance) of each standard on a log-log scale. The use of software to generate a cubic spline fit curve is recommended.

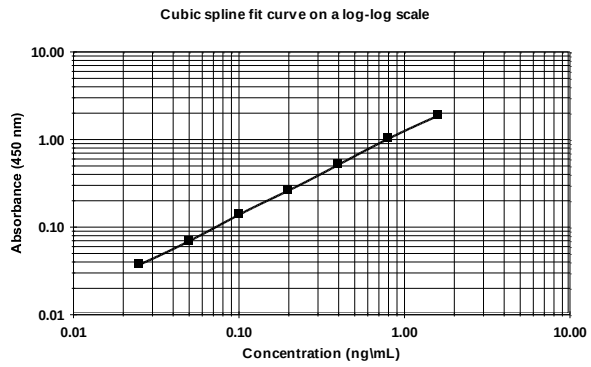
The human CD48 concentration in the sample can be calculated by interpolation between standard points on the curve.

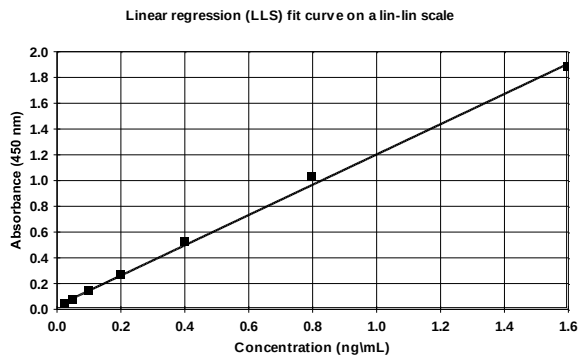
When generating a linear regression fit curve instead of a cubic spline fit curve only minor differences occur in human CD48 concentration calculation.

Validation of the assay: The mean absorbance of the Standard A (blank) should be below 0.1 AU (absorbance unit). The mean absorbance of the Standard H is usually above 1.0 AU.

TYPICAL DATA

These standard curves are shown as an example of a typical assay (Not to be used for calculation of actual test results).





PRECISION

Intra-assay precision:

Sample	Number of measures	Mean (pg/mL)	CV%
1	16	1380	2.4
2	16	420	3.2
3	16	170	2.9
4	16	100	4.2

Inter-assay precision:

Sample	Number of assays	Mean (pg/mL)	CV%
1	3	1293	6.97
2	3	410	2.44
3	3	173	3.33
4	3	100	0

LINEARITY TEST (HUMAN SERUM, PLASMA)

Four samples were diluted with sample diluent. The concentration of human CD48 in each diluted sample was measured. The results are shown as a change in percentage from the lowest dilution (corrected with the dilution factor).

Sample	Dilution	Conc. (pg\mL)	%
Serum #1	4	3930	100
	8	4290	109
	16	4410	112
	32	4180	106
Serum #2	4	5950	100
	8	6940	117
	16	6890	116
	32	6090	102
Plasma #1	4	4030	100
	8	4300	107
	16	4300	107
	32	4130	103
Plasma #2	4	3200	100
	8	3420	107
	16	3510	110
	32	3250	102

LINEARITY TEST (CELL CULTURE SUPERNATANT)

One sample was diluted with sample diluent. The concentration of human CD48 in the diluted sample was measured. The results are shown as a change in percentage from the lowest dilution (corrected with the dilution factor).

Sample	Dilution	Conc. (ng\mL)	%
Cell culture supernatant #1	25 000	33 000	100
	50 000	31 750	96
	100 000	32 210	98
	500 000	29 050	88

RECOVERY (SERUM, PLASMA)

Human CD48 standards of 25, 100, 400 and 1600 pg/mL were added to equal volumes of four samples containing a low (100 pg\mL), a medium (420 pg\mL) and a high (1380 pg\mL) concentration of human CD48. The theoretical concentration and the recovered concentration were calculated.

Sample	Added conc. (pg/mL)	Expected conc. (pg/mL)	Obtained conc. (pg/mL)	Recovery %
Low	0		100	100
	25	62.5	70	112
	100	100	110	110
	400	250	260	104
	1600	850	870	102
Medium	0		420	100
	25	222.5	240	108
	100	260	290	112
	400	410	450	110
	1600	1010	1040	103
High	0		1380	100
	25	702.5	790	112
	100	740	840	114
	400	890	980	110
	1600	1490	1680	113

RECOVERY (CELL CULTURE SUPERNATANT)

Human CD48 standards of 50, 200, 800 and 1600 pg/mL were added to equal volumes of one sample (cell culture supernatant) containing a medium (320 pg\mL) concentration of human CD48. The theoretical concentration and the recovered concentration were calculated.

Sample	Added conc. (pg/mL)	Expected conc. (pg/mL)	Obtained conc. (pg/mL)	Recovery %
Medium	0		320	100
	50	185	190	103
	200	260	260	100
	800	560	560	100
	1600	960	980	102

TECHNICAL ASSISTANCE

Please refer any technical questions to technical.support@icosagen.com.