MOUSE CD48 QUANTIFICATION KIT

APPLICATION

The mouse CD48 Quantification kit provides a rapid and easy method for the quantitative determination of mouse CD48 in cell culture supernatant and serum. The kit includes ready-to-use reagents necessary to analyse up to 88 samples in 2 hours and 30 minutes.

PRINCIPLE OF THE ASSAY

The mouse CD48 test is based on the quantitative sandwich enzyme immunoassay technique. Microtiter wells are pre-coated with mouse CD48-specific polyclonal capture antibodies. Samples and standards are pipetted into microwells and mouse 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 mouse CD48-specific polyclonal detection antibodies bind to different epitopes of mouse 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 mouse 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 50 pg/mL to 3200 pg/mL.
The detection limit is 6 pg/mL to 16 pg/mL, defined by the minimum mouse 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-mouse CD48 rabbit polyclonal antibodies.
- Mouse CD48 sample diluent, 25 mL, pink solution (PBS pH7.4, BPLA, detergent and preservative)
- Mouse CD48 standards A-H, 1 mL, pink solution (0-50-100-200-400-800-1600-3200 pg/mL)
- Mouse CD48 enzyme conjugate stock, 30 µL, (HRP-conjugated rabbit polyclonal antibodies in a stabilizing solution)
- Mouse 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) (1:500)
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 60 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 25 minutes 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 (1:500). Discard any remaining working solution after use.

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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.

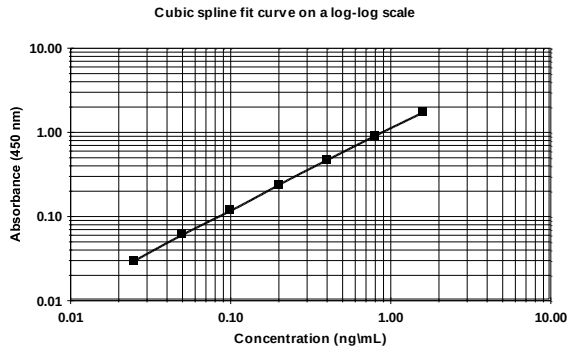
The mouse CD48 concentration in the sample can be calculated by interpolation between standard points on the curve.

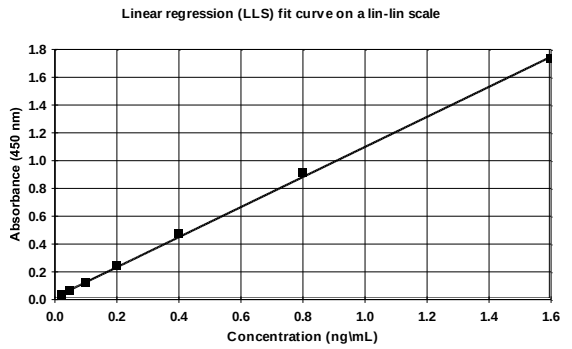
When generating a linear regression fit curve instead of a cubic spline fit curve only minor differences occur in mouse CD48 concentration calculation.

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 1.5 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 (ng/mL)	CV%
1	8	10.2	2.4
2	8	19.6	3.3

Inter-assay precision:

Sample	Number of assays	Mean (ng/mL)	CV%
1	3	10.5	3.8
2	3	20.6	4.8

LINEARITY TEST (MOUSE SERUM)

Two samples were diluted with sample diluent. The concentration of mouse 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. (ng\mL)	%
Serum #1	4	10.9	100
	8	10.6	97
	16	10.1	92
	32	9.3	85
Serum #2	20	20.9	100
	40	20.8	99
	80	16.5	79
	160	19.2	91

LINEARITY TEST (CELL CULTURE SUPERNATANT)

One sample was diluted with sample diluent. The concentration of mouse 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	30 376	100
	50 000	28 669	94
	100 000	29 424	97

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RECOVERY (MOUSE SERUM)

Mouse CD48 standards of 25, 100, 400 and 1600 pg/mL were added to equal volumes of three samples containing a low (40 pg\mL), a medium (490 pg\mL) and a high (1280 pg\mL) concentration of mouse 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		40	100
	25	-	-	-
	100	70	70	100
	400	220	220	100
	1600	820	800	98
Medium	0		490	100
	25	257.5	230	89
	100	295	260	88
	400	445	390	88
	1600	1045	980	94
High	0		1280	100
	25	652.5	590	90
	100	690	640	93
	400	840	760	90
	1600	1440	1330	92

RECOVERY (CELL CULTURE SUPERNATANT)

Mouse CD48 standards of 50, 200, 800 and 1600 pg/mL were added to equal volumes of one sample (cell culture supernatant) containing a medium (290 pg\mL) concentration of mouse 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		290	100
	50	170	170	100
	200	245	250	102
	800	545	540	99
	1600	945	960	102

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