HUMAN MANF QUANTIFICATION KIT

APPLICATION

The human MANF Quantification kit provides a rapid and easy method for the quantitative determination of human MANF in cell culture supernatant, serum and plasma. The kit includes ready-to-use reagents necessary to analyse up to 88 samples in 2 and a half hours.

PRINCIPLE OF THE ASSAY

The human MANF test is based on the quantitative sandwich enzyme immunoassay technique. Microtiter wells are pre-coated with human MANF-specific monoclonal capture antibodies. Samples and standards are pipetted into microtiter plate wells and human MANF 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 MANF-specific polyclonal detection antibodies bind to different epitopes of human MANF 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 MANF 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 0.25 ng/mL to 16 ng/mL.
The detection limit is 30 pg/mL to 75 pg/mL, defined by the minimum human MANF 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 (microplate desiccation recommended).

KIT CONTENTS

- Pre-coated microtiter plate: 96 wells coated with anti-human MANF mouse monoclonal antibodies.
- Human MANF sample diluent, 25 mL, pink solution (PBS pH7.4, BPLA, detergent and preservative)
- Human MANF standards A-H, 1 mL, pink solution (0-0.25-0.5-1-2-4-8-16 ng/mL)
- Human MANF enzyme conjugate stock, 80 µl (HRP-conjugated chicken polyclonal antibodies in a stabilizing solution)
- Human MANF 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 (1-100 µl)
- Microplate reader (450 nm)
- Lid or sealing tape for microtiter plate
- Microtiter 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 into appropriate wells in duplicate.
STEP 4	Incubate the covered microtiter plate for 60 min at RT on a microplate shaker (300 rpm).
STEP 5	Prepare enzyme conjugate working solution by diluting enzyme conjugate stock in enzyme conjugate stock diluent (blue) (1:150)
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 microtiter plate for 60 min at RT on a microplate 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 microtiter plate for 20 minutes at RT on a microtiter 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 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 (1:150). Discard any remaining working solution after use.

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MICROWELL PLATE WASH

Manual wash is recommended (e.g. using a multi-channel pipette) during the washing steps, as a microplate 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 absorbance. 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.

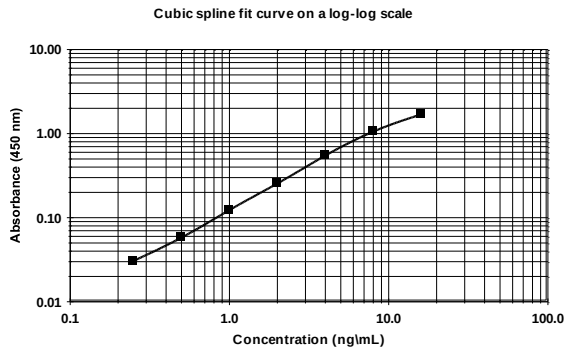
The human MANF concentration in the sample can be calculated by interpolation between standard points on the curve.

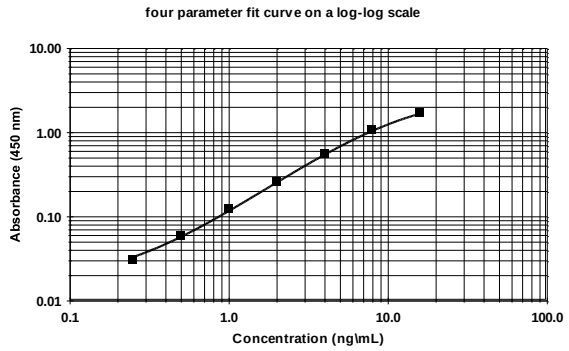
When generating a four-parameter logistic fit curve instead of a cubic spline fit curve only minor differences occur in human MANF concentration calculation.

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 1.4 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%
Plasma #1	16	6.37	4.48
Plasma #2	16	2.13	4.77
Serum #1	16	1.02	7.84
Serum #2	16	2.64	3.71

Inter-assay precision:

Sample	Number of assays	Mean (ng/mL)	CV%
Plasma #1	4	7.1	9.9
Plasma #2	4	2.3	6.2
Serum #1	4	1.1	3.0
Serum #2	4	2.5	3.9

LINEARITY (DILUTION TEST)

Four samples (human plasma or serum) were diluted with sample diluent. The concentration of human MANF 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 factor	Conc. (ng\mL)	%
Plasma #1	16	46.6	100
	32	48.07	103
	64	48.25	104
Plasma #2	16	15.52	100
	32	17.8	115
	64	17.14	110
Serum #1	4	5.19	100
	8	5.36	103
	16	5.86	113
Serum #2	4	12.9	100
	8	14.64	114
	16	15.39	119

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RECOVERY (HUMAN)

Human MANF standards of 0.25, 1 and 4 ng/mL were added to equal volumes of four samples (human plasma or serum). The theoretical concentration and the recovered concentration were calculated.

Sample	Added conc. (ng/mL)	Expected conc. (ng/mL)	Obtained conc. (ng/mL)	Recovery %
#1	0		4.7	100
	0.25	2.5	2.5	103
	1	2.8	2.9	101
	4	4.3	4.0	91
#2	0		6.5	100
	0.25	3.4	4.0	119
	1	3.8	4.2	113
	4	5.3	5.5	104
#3	0		1.3	100
	0.25	0.8	0.9	108
	1	1.2	1.2	99
	4	2.7	2.3	87
#4	0		1.4	100
	0.25	0.8	0.9	108
	1	1.2	1.2	99
	4	2.7	2.4	90

RECOVERY (MOUSE AND RABBIT)

Human MANF standards of 0.25, 1, 4 and 16 ng/mL were added to equal volumes of two samples (mouse and rabbit serum). The theoretical concentration and the recovered concentration were calculated.

Sample	Added conc. (ng/mL)	Expected conc. (ng/mL)	Obtained conc. (ng/mL)	Recovery %
mouse serum	0		UD	
	0.25	0.125	UD	
	1	0.5	0.51	102
	4	2	1.94	97
	16	8	6.93	87
rabbit serum	0		UD	
	0.25	0.125	UD	
	1	0.5	0.53	106
	4	2	1.98	99
	16	8	7.01	88

UD - Undetectable

TECHNICAL ASSISTANCE

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

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