HUMAN ARTEMIN QUANTIFICATION KIT

APPLICATION

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

PRINCIPLE OF THE ASSAY

The hARTN test is based on the quantitative sandwich enzyme immunoassay technique. Microtiter wells are pre-coated with hARTN-specific monoclonal capture antibodies. Samples and standards are pipetted into microwells and hARTN 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 hARTN-specific monoclonal detection antibodies bind to the same epitope on another molecule of dimeric hARTN. After washing, the ready-to-use HRP substrate (TMB) is added to the wells. The intensity of the colour produced is directly proportional to the amount of hARTN in the sample. Colour development is then stopped by the addition of stop solution. Absorbance is measured at 450 nm.

SENSITIVITY

The detection range of the assay is from 30 pg/mL to 1920 pg/mL. The detection limit is 2 pg/mL to 8 pg/mL, defined by the minimum hARTN concentration deviating by 2 SD (Standard Deviation) from that of the Standard A. The test was performed by using 16 replicate determinations of the Standard A (blank) and Standard B.

STORAGE CONDITIONS

The kit should be stored at +2...+6°C. The unopened kit is 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).

As hARTN may be unstable in low concentrations (diluted standard stock solution, standards and diluted samples) it is recommended to prepare the standards and samples directly before the test procedure.

hARTN standards can be stored at -20°C up to one week if frozen immediately after preparation. Thaw and use only once.

KIT CONTENTS

- Pre-coated microwell plates: 96 microwells coated with hARTN-specific mouse monoclonal antibodies
- hARTN sample diluent, 25 mL, pink solution
  (PBS pH 7.4, BPLA, detergent and preservative)
- hARTN standard stock solution, 2 × 0.05 mL, pink solution (1.92 µg/mL)
- hARTN-specific enzyme conjugate, 12 mL, blue solution
  HRP-conjugated mouse monoclonal antibody in a buffered solution containing 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 (10−1000 µl)
- (Micro)centrifuge tubes
- 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) for 30 minutes. 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 wash concentrate with 450 mL of distilled water.
STEP 2	Prepare the standards directly before the test by serial dilutions using the stock standard and the sample diluent (pink).
STEP 3	Perform the dilution of each sample in the sample diluent directly before the test.
STEP 4	Add 100 µL samples and standards into appropriate wells in duplicate.
STEP 5	Incubate the covered microplate for 60 min at RT on a microwell plate shaker (300 rpm).
STEP 6	Remove the liquid and wash the wells 4 times with 300 µL of washing solution.
STEP 7	Add 100 µL of enzyme conjugate (blue) into each well.
STEP 8	Incubate the covered microplate for 30-60 min at RT on a microwell plate shaker (300 rpm).
STEP 9	Remove the liquid 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 20 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.

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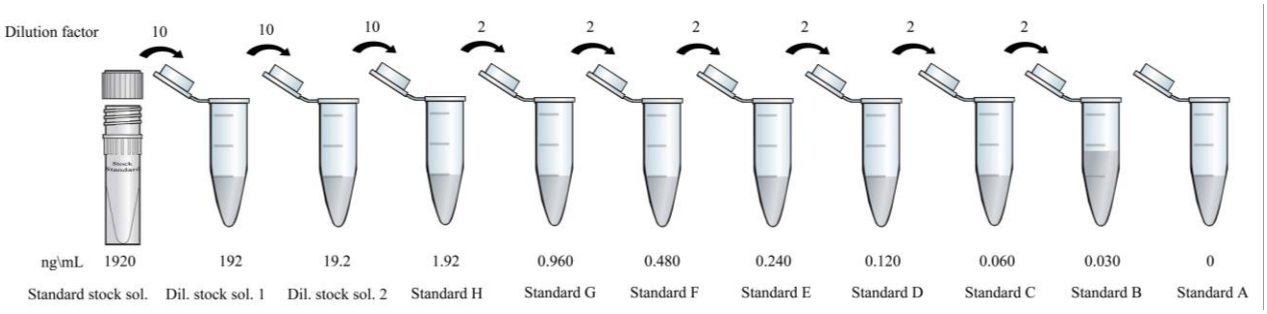
PREPARATION OF STANDARDS



Prepare the standards by serial dilution of the standard stock solution in the sample diluent according to the table below. The volumes of the standards given in the table are calculated for one assay (standard curve) only.

Standard	Concentration of hARTN	Vol. of hARTN solution (µL)	Vol. of sample diluent (µL)
Standard stock solution	1.92 µg\ml		
Diluted stock solution 1	192 ng\ml	10 µl of standard stock solution	90 µl
Diluted stock solution 2	19.2 ng\ml	10 µl of diluted stock sol. 1	90 µl
H	1.92 ng\ml	50 µl of diluted stock sol. 2	450 µl
G	960 pg\ml	250 µl of standard H	250 µl
F	480 pg\ml	250 µl of standard G	250 µl
E	240 pg\ml	250 µl of standard F	250 µl
D	120 pg\ml	250 µl of standard E	250 µl
C	60 pg\ml	250 µl of standard D	250 µl
B	30 pg\ml	250 µl of standard C	250 µl
A	0 pg\ ml		250 µl

MICROWELL PLATE WASH

It is recommended to wash the microwell plate wells manually (e.g. using a multi-channel pipette) during the washing steps, as a plate washer may cause poor precision.

CALCULATION OF RESULTS

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

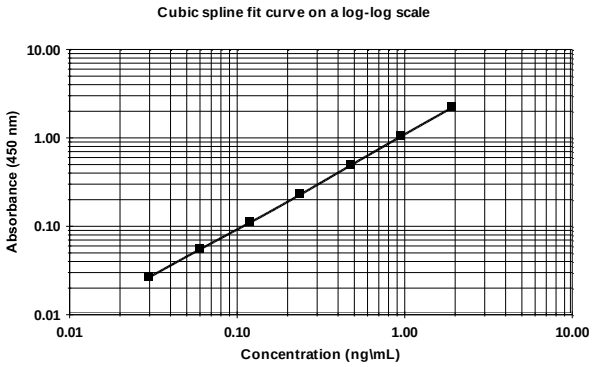
The hARTN concentration in the sample can be calculated by interpolation between standard points on the curve.

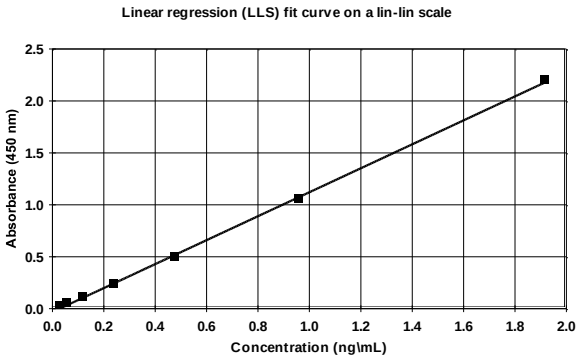
When generating a linear regression fit curve instead of a cubic spline fit curve only minor differences occur in calculated hARTN concentration.

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 1.9 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).





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PRECISION

Intra-assay precision:

Sample	Number of measures	Mean (pg/mL)	CV%
1	8	100	1.99
2	8	460	1.23
3	8	1830	4.57

Inter-assay precision:

Sample	Number of assays	Mean (pg/mL)	CV%
1	3	110	9.09
2	3	450	8.91
3	3	1910	5.54

LINEARITY (DILUTION TEST)

Four samples (plasma or serum) were diluted with sample diluent. The concentration of hARTN 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. (pg\mL)	%
Serum #1	4	3070	100
	8	2800	91.2
	16	2830	92.2
	32	3000	97.7
Serum #2	4	670	100
	8	620	92.5
	16	550	82.1
Plasma #1	4	3030	100
	8	2800	92.4
	16	2820	93.1
	32	3050	100.7
Plasma #2	4	580	100
	8	560	96.6
	16	570	98.3

RECOVERY

hARTN standards of 30, 120 and 960 pg/mL were added to equal volumes of two samples containing a high (1590 pg/mL) and low (40 pg/mL) concentration of hARTN. The theoretical concentration and the recovered concentration were calculated.

Sample	Added conc. (pg/mL)	Expected conc. (pg/mL)	Obtained conc. (pg/mL)	Recovery %
1	0		1590	100
	30	810	1000	123
	120	855	1000	117
	960	1275	1490	117
2	0		40	100
	30	50	40	80
	120	80	90	113
	960	500	560	112