Version 22-04/24

User's Manual

Human Artemin ELISA Kit

REF **DEIASL192**

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RUO

This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

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PRODUCT INFORMATION

Intended Use

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.

Principles of Testing

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 hARTNspecific 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.

Reagents And Materials Provided

1. Pre-coated microwell plates: 96 microwells coated with hARTN-specific mouse monoclonal antibodies
2. hARTN sample diluent, 25 mL, pink solution (PBS pH 7.4, BPLA, detergent and preservative)
3. hARTN standard stock solution, 2 x 0.03 mL, pink solution (1.92 µg/mL)
4. hARTN-specific enzyme conjugate, 12 mL, blue solution. HRP-conjugated mouse monoclonal antibody in a buffered solution containing BPLA, detergent and preservative.
5. Wash concentrate, 50 mL. (PBS pH 7.4 and detergent)
6. Substrate solution (TMB), 12 mL
7. Stop solution (0.5 M H_2SO_4), 12 mL

Materials Required But Not Supplied

1. Pipettes and tips (10−1000 µl)
2. (Micro)centrifuge tubes
3. Microplate reader (450 nm)
4. Lid or sealing tape for microwell plate
5. Microwell plate shaker

Storage

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).

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

Specimen Collection And Preparation

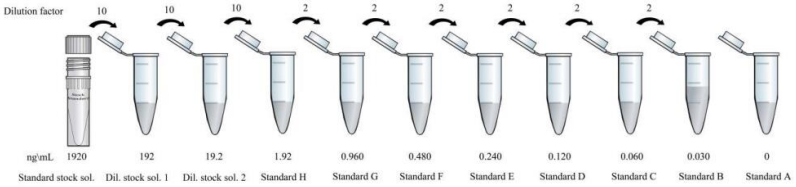
Dilute the samples in sample diluent.

Reagent Preparation

PREPARATION OF STANDARDS

Prepare the standards immediately prior to use by serial dilution according to the table below. Try to avoid vortex in the process. Pipette up and down to mix gently. Avoid foaming or bubbles when mixing components. The volumes of the standards given in the table are calculated for one assay (standard curve) only.

Standard	Conc. 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



Assay Procedure

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

1. Dilute 50 mL of wash concentrate with 450 mL of distilled water to prepare washing solution.
2. Prepare standards directly before use by serial dilution using stock standard and sample diluent (pink).
3. Perform dilutions of each sample in sample diluent directly before use.
4. Add 100 µL of samples and standards (pink) into appropriate wells in duplicate.
5. Incubate the covered microplate for 60 min at RT on a microwell plate shaker (300 rpm).
6. Discard the solution and wash the wells 4 times with 300 µL of washing solution. 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.
7. Add 100 µL of enzyme conjugate (blue) into each well.
8. Incubate the covered microplate for 30 min at RT on a microwell plate shaker (300 rpm).
9. Discard the solution and wash the wells 4 times with 300 µL of washing solution.
10. Add 100 µL of substrate solution into each well.
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).
12. Stop the reaction by adding 50 µl of STOP solution into each well in the same order and time as for TMB distribution.
13. Read the absorbance at 450 nm immediately.

Quality Control

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.

Calculation

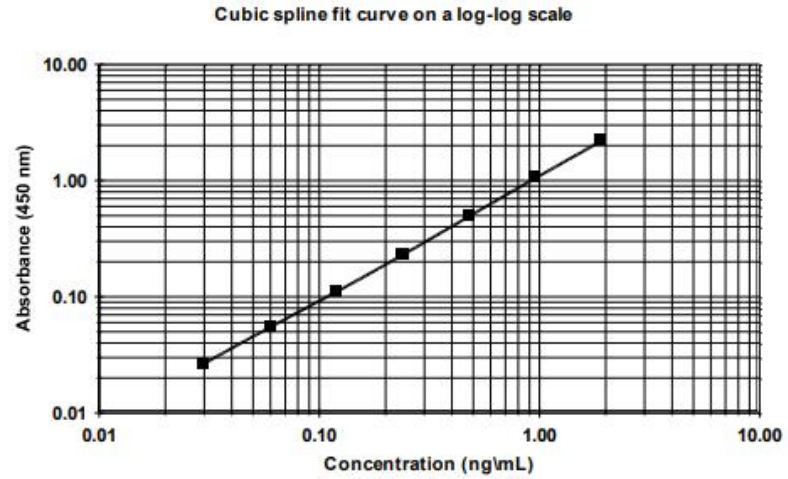
Calculate the mean absorbance for each standard. Subtract the blank value (standard A) from the mean absorbances. Plot the value (absorbance) of each standard on a log-log scale. The use of software to generate a cubic spline fit curve is recommended.

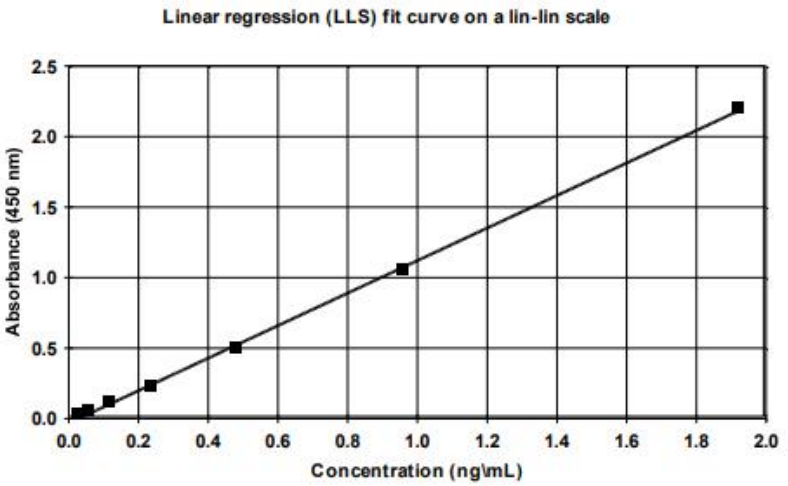
The hARTN concentration in the sample can be calculated by interpolation between standard points on the curve.

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

Typical Standard Curve

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	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

Detection Range

The detection range of the assay is from 30 pg/mL to 1920 pg/mL.

Detection Limit

The detection limit is 2 pg/mL to 8 pg/mL, defined by the minimum hARTN 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.

Linearity

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
	16	2830	92
	32	3000	98
Serum #2	4	670	100
	8	620	93
	16	550	82
Plasma #1	4	3030	100
	8	2800	92
	16	2820	93
	32	3050	101
Plasma #2	4	580	100
	8	560	97
	16	570	98

Recovery

hARTN standards of 30, 120 and 960 pg/mL were added to equal volumes of two samples (serum or plasma) containing a low (40 pg/mL) and a high (1590 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 %
Low	0		40	100
	30	50	40	80
	120	80	90	113
	960	500	560	112
High	0		1590	100
	30	810	1000	124
	120	855	1000	117
	960	1275	1490	117