HUMAN RNASE 7 QUANTIFICATION KIT
INSTRUCTION MANUAL

APPLICATION

The human RNase 7 Quantification kit provides a rapid and easy method for the quantitative determination of human RNase 7 (all three natural variants) in biological fluids (urine, saliva, skin lavage etc.) and cell culture supernatant. 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 RNase 7 test is based on the quantitative sandwich enzyme immunoassay technique. Microtiter wells are pre-coated with human RNase 7-specific monoclonal capture antibodies. Samples and standards are pipetted into microwells and human RNase 7 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 RNase 7-specific monoclonal detection antibodies bind to a different epitope of human RNase 7 molecules. 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 human RNase 7 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 10 pg/mL to 640 pg/mL. The detection limit is from 1 pg/mL to 6 pg/mL, defined by the minimum human RNase 7 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 human RNase 7-specific mouse monoclonal antibodies
- Human RNase 7 sample diluent (Citrate buffer, BPLA, detergent and preservative), 25 mL, pink solution
- Human RNase 7 standard stock solution (640 ng/mL), 2 × 30 µL, pink solution
  The canonical form of human RNase 7 (Uniprot ID Q9H1E1) is used as the standard
- Human RNase 7 enzyme conjugate (HRP-conjugated mouse monoclonal antibody in a buffered solution containing BPLA, detergent and preservative), 12 mL, blue solution
- Wash concentrate (PBS pH 7.4 and detergent), 50 mL
- 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) 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.

STEP 1	Dilute 50 mL of wash concentrate with 450 mL of distilled water to prepare washing solution.
STEP 2	Prepare standards by serial dilution using stock standard and sample diluent (pink).
STEP 3	Perform dilutions of each sample in sample diluent.
STEP 4	Add 100 µL of samples and standards (pink) 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	Discard the solution 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 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 10 - 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.

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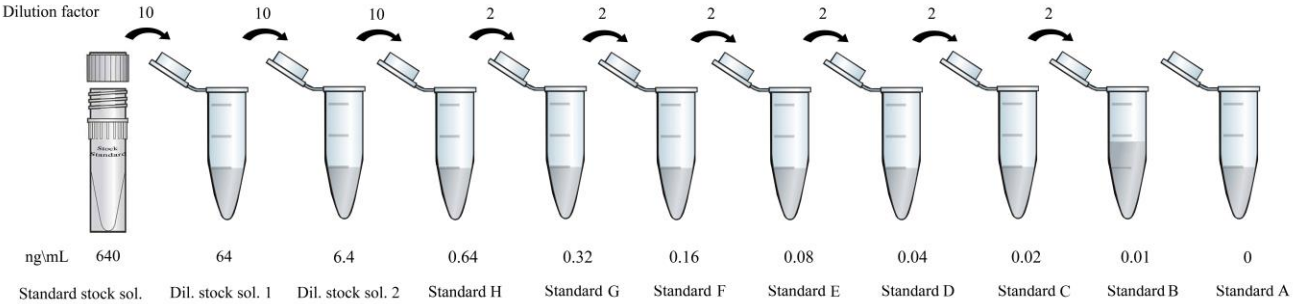
PREPARATION OF STANDARDS



Prepare the standards by serial dilution according to the table below. Avoid foaming or bubbles when mixing components. The volumes of the standards given in the table are calculated for one assay (standard curve) only. It is recommended to prepare standards and samples directly before the test procedure. Discard any remaining standards after use.

Standard	Concentration of hRNase 7	Vol. of hRNase 7 solution (µL)	Vol. of sample diluent (µL)
Stock solution	640 ng\ml		
Diluted stock solution 1	64 ng\ml	10 µl of stock solution	90 µl
Diluted stock solution 2	6.4 ng\ml	10 µl of diluted stock sol. 1	90 µl
H	640 pg\ml	50 µl of diluted stock sol. 2	450 µl
G	320 pg\ml	250 µl of standard H	250 µl
F	160 pg\ml	250 µl of standard G	250 µl
E	80 pg\ml	250 µl of standard F	250 µl
D	40 pg\ml	250 µl of standard E	250 µl
C	20 pg\ml	250 µl of standard D	250 µl
B	10 pg\ml	250 µl of standard C	250 µl
A	0 pg\ ml		250 µl

PREPARATION OF SAMPLES

Dilute the samples in sample diluent.

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.

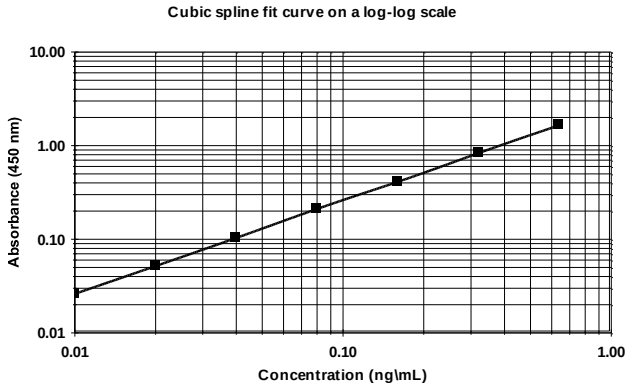
The human RNase 7 concentration in the sample can be calculated by interpolation between standard points on the curve.

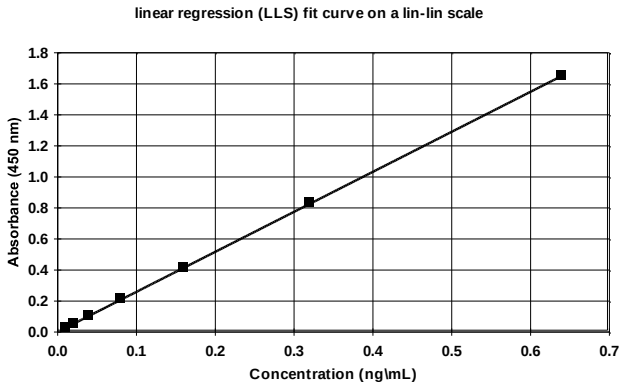
When generating linear regression fit curve instead of cubic spline fit curve only minor differences occur in human RNase 7 concentration calculation.

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 1.3 AU.

TYPICAL DATA

These standard curves are shown as an example of 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%
Urine #1	16	30	4.4
Urine #2	16	220	2.8

Inter-assay precision:

Sample	Number of assays	Mean (pg/mL)	CV%
Urine #1	5	20	0
Urine #2	5	234	6.48

LINEARITY (DILUTION TEST)

Two samples of urine were diluted with sample diluent. The concentration of human RNase 7 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)	%
Urine #1	2	930	100
	4	940	101
	8	870	94
	16	920	99
	32	780	84
Urine #2	8	3460	100
	16	3410	99
	32	3250	94
	64	3240	94
	128	3410	99

RECOVERY

Human RNase 7 standards of 10, 40, 80 and 160 pg/mL were added to equal volumes of two samples containing a low (30 pg/mL) and a high (220 pg/mL) concentration of human RNase 7. 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		30	100
	10	20	20	100
	40	35	30	86
	80	55	50	91
	160	95	90	95
High	0		220	100
	10	115	100	87
	40	130	120	92
	80	150	140	93
	160	190	190	100

TECHNICAL ASSISTANCE

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