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ab215418
Human RNAse 7 ELISA kit

For the quantitative determination of human RNAse in biological fluids (urine, saliva, skin lavage etc.) and cell culture supernatant

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

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1. Overview

The human RNAse 7 ELISA kit (ab215418) 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 analyze up to 88 samples in 2 and a half hours.

The human RNAse 7 test is based on the quantitative sandwich enzyme immunoassay technique. Microplate wells are pre-coated with anti-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 color produced is directly proportional to the amount of human RNAse 7 in the sample. Color development is then stopped by the addition of stop solution. Absorbance is measured at 450 nm.

2. Protocol Summary

Prepare all reagents, samples, and standards as instructed.

Add 100 µL of samples and standards into appropriate wells.

Incubate for 1 hour at RT. Discard the solution and wash the wells four times with 300 µL of washing solution.

Add 100 µL of enzyme conjugate into each well.

Incubate for 1 hour at RT. Discard the solution and wash the wells 4 times with 300 µL of washing solution.

Add 100 µL of substrate solution into each well.

Incubate for 10 - 25 minutes (the precise incubation time comes with the kit) at RT.

Add 50 µL of Stop solution into each well. Read the absorbance at 450 nm immediately.

3. Precautions

Please read these instructions carefully prior to beginning the ELISA assay.

- All kit components have been formulated and quality control tested to function successfully as a kit.
- We understand that, occasionally, experimental protocols might need to be modified to meet unique experimental circumstances. However, we cannot guarantee the performance of the product outside the conditions detailed in this protocol booklet.
- Reagents should be treated as possible mutagens and should be handled with care and disposed of properly. Please review the Safety Datasheet (SDS) provided with the product for information on the specific components.
- Observe good laboratory practices. Gloves, lab coat, and protective eyewear should always be worn. Never pipet by mouth. Do not eat, drink or smoke in the laboratory areas.
- All biological materials should be treated as potentially hazardous and handled as such. They should be disposed of in accordance with established safety procedures.

4. Storage and Stability

Store ELISA kit at 2-8°C immediately upon receipt. Kit has a storage time of 1 year from receipt, providing components have not been reconstituted.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in the Materials Supplied section.

Aliquot components in working volumes before storing at the recommended temperature.

5. Limitations

- ELISA kit intended for research use only. Not for use in diagnostic procedures.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

6. Materials Supplied

Item	Quantity	Storage Condition (Before prep)	Storage Condition (After prep)
Anti-Human RNase 7 coated microplate (12 x 8 wells)	96 well	+2-8°C	+2-8°C
Human RNase 7 sample diluent	25 mL	+2-8°C	+2-8°C
Human RNase 7 standard stock solution	2 x 30 µL	+2-8°C	+2-8°C
Human RNase 7 enzyme conjugate	12 mL	+2-8°C	+2-8°C
10X Wash concentrate	50 mL	+2-8°C	+2-8°C
TMB Substrate solution	12 mL	+2-8°C	+2-8°C
Stop solution	12 mL	+2-8°C	+2-8°C

7. Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully perform this assay:

- Pipettes and tips (10-1000 µL)
- (Micro)centrifuge tubes
- Microplate reader (450 nm)
- Lid or sealing tape for microwell plate
- Microwell plate shaker

8. Technical Hints

- Samples generating values higher than the highest standard should be further diluted in the appropriate sample dilution buffers.
- Avoid foaming or bubbles when mixing or reconstituting components.
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions.
- Ensure plates are properly sealed or covered during incubation steps.
- 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.
- Complete removal of all solutions and buffers during wash steps is necessary to minimize background.
- All samples should be mixed thoroughly and gently.
- Avoid multiple freeze/thaw of samples.
- When generating positive control samples, it is advisable to change pipette tips after each step.
- This kit is sold based on number of tests. A 'test' simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.

9. Reagent Preparation

- Equilibrate all reagents to room temperature (18-25°C) prior to use. The kit contains enough reagents for 96 wells.
- Prepare only as much reagent as is needed on the day of the experiment.

9.1 Anti-Human RNase 7 coated microplate (12 x 8 wells)

96 well microtiter plate coated with anti-human RNAse 7-specific mouse monoclonal antibodies. 96 tests. Ready to use. Store at +2-8°C.

9.2 Human RNase 7 sample diluent

25 mL. Ready to use. Store at +2-8°C.

9.3 Human RNase 7 standard stock solution

2 tubes of 30 µL. Store at +2-8°C. The canonical form of human RNase 7 (Uniprot ID Q9H1E1) is used as the standard.

9.4 Human RNase 7 enzyme conjugate

12 mL. Ready to use. Store at +2-8°C.

9.5 10X Wash concentrate

50 mL. Store at +2-8°C. Dilute 50 mL of 10X Wash concentrate with 450 mL of distilled water to prepare 1X Washing solution.

9.6 TMB Substrate solution

12 mL. Ready to use. Store at +2-8°C.

9.7 Stop solution

12 mL. Ready to use. Store at +2-8°C.

10. Standard Preparation

10.1 Prepare the standards by serial dilution according to the table below.
10.2 Avoid foaming or bubbles when mixing components.
10.3 The volumes of the standards given in the table are calculated for one assay (standard curve) only.
10.4 Human RNase 7 prepared standards can be stored at +2-8°C up to one week

Standard	Concentration of Human RNase 7	Volume of Human RNase 7 (µL)	Volume of sample diluent (µL)
Standard stock solution	640 ng/mL	N/A	N/A
Diluted stock solution 1	64 ng/mL	10 µL of stock solution	90
Diluted stock solution 2	6.4 ng/mL	10 µL of diluted stock solution 1	90
H	640 pg/mL	50 µL of diluted stock solution 2	450
G	320pg/mL	250 µL of standard H	250
F	160 pg/mL	250 µL of standard G	250
E	80pg/mL	250 µL of standard F	250
D	40 pg/mL	250 µL of standard E	250
C	20 pg/mL	250 µL of standard D	250
B	10 pg/mL	250 µL of standard C	250
A	0 pg/mL	N/A	250

11. Sample Preparation

11.1 Samples

Dilute the samples in sample diluent.

12. Plate Preparation

- The 96 well plate strips included with this kit are supplied ready to use. It is not necessary to rinse the plate prior to adding reagents.
- For statistical reasons, we recommend each sample should be assayed with a minimum of two replicates (duplicates).
- Differences in well absorbance or "edge effects" have not been observed with this assay.

13. Assay Procedure

- Equilibrate all materials and prepared reagents to room temperature prior to use.
- It is recommended to assay all standards, controls and samples in duplicate.
- 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.

13.1 Add 100 µL of samples and standards into appropriate wells in duplicate.
13.2 Incubate the covered microplate for 1 hour at RT on a microwell plate shaker (300 rpm).
13.3 Discard the solution and wash the wells 4 times with 300 µL of washing solution.
13.4 Add 100 µL of enzyme conjugate into each well.
13.5 Incubate the covered microplate for 1 hour at RT on a microwell plate shaker (300 rpm).
13.6 Discard the solution and wash the wells 4 times with 300 µL of washing solution.
13.7 Add 100 µL of substrate solution into each well.
13.8 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).
13.9 Stop the reaction by adding 50 µL of Stop solution into each well in the same order and time as for TMB distribution.
13.10 Read the absorbance at 450 nm immediately.

14. Calculations

14.1 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.

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

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

14.4 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 1.0 AU.

15. Typical data

Typical standard curve – Data provided for demonstration purposes only. A new standard curve must be generated for each assay performed.

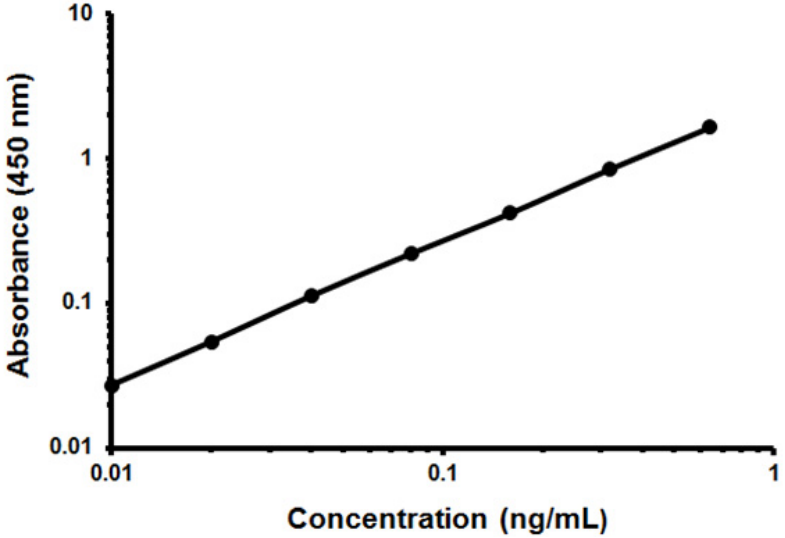

Figure 1. Cubic spline fit curve on a log-log scale

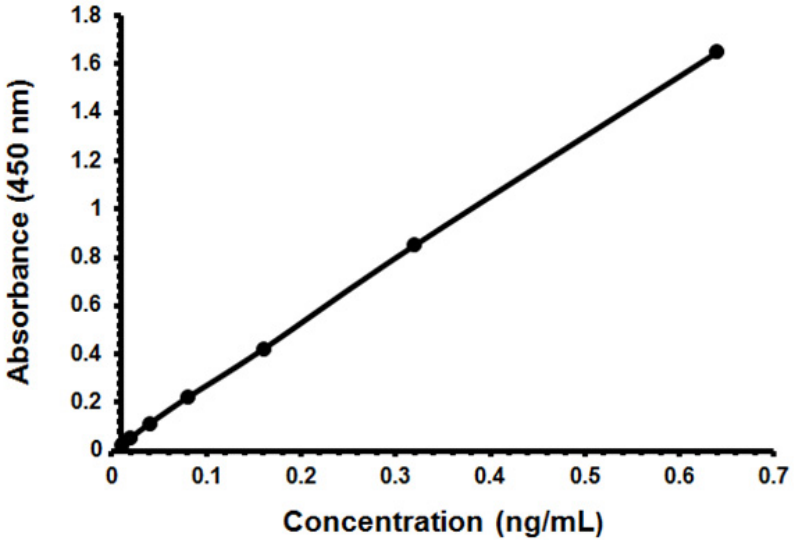

Figure 2. Linear regression (LLS) fit curve on a lin-lin scale

16. Typical sample values

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.

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	N/A	30	100
	10	20	20	100
	40	35	30	86
	80	55	50	91
	160	95	90	95
High	0	N/A	220	100
	10	115	100	87
	40	130	120	92
	80	150	140	93
	160	190	190	100

LINEARITY OF DILUTION –

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	Conc. (pg/mL)	%
Urine #1	1:2	930	100
	1:4	940	101
	1:8	870	94
	1:16	920	99
	1:32	780	84
Urine #2	1:8	3460	100
	1:16	3410	99
	1:32	3250	94
	1:64	3240	94
	1:128	3410	99

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

17. Troubleshooting

Problem	Cause	Solution
Poor standard curve	Inaccurate Pipetting	Check pipettes
	Improper standard dilution	Prior to opening, briefly spin the stock standard tube and dissolve the powder thoroughly by gentle mixing
Low Signal	Incubation times too brief	Ensure sufficient incubation times standard/sample incubation
	Inadequate reagent volumes or improper dilution	Check pipettes and ensure correct preparation
	Incubation times with TMB too brief	Ensure sufficient incubation time until blue color develops prior addition of Stop solution
Large CV	Plate is insufficiently washed	Review manual for proper wash technique. If using a plate washer, check all ports for obstructions.
	Contaminated wash buffer	Prepare fresh wash buffer
Low sensitivity	Improper storage of the ELISA kit	All components 4°C. Keep TMB substrate solution protected from light.

18. Notes

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