Product Information

Human ENA78/CXCL5 ELISA Kit

RAB0130

Product Description

The Human ENA78/CXCL5 ELISA kit is an in vitro enzyme-linked immunosorbent assay for the quantitative measurement of human ENA-78 in serum, plasma, and cell culture supernatants. This assay employs an antibody specific for human ENA-78 coated on a 96-well plate. Standards and samples are pipetted into the wells and ENA-78 present in a sample is bound to the wells by the immobilized antibody. The wells are washed and biotinylated anti-human ENA-78 antibody is added. After washing away unbound biotinylated antibody, HRP-conjugated streptavidin is pipetted to the wells. The wells are again washed, a TMB substrate solution is added to the wells and color develops in proportion to the amount of ENA-78 bound. The Stop Solution changes the color from blue to yellow, and the intensity of the color is measured at 450 nm.

Components

- Human ENA78/CXCL5 Antibody-coated ELISA Plate (Item A)-RAB0130A: 96-wells (12 strips x 8 wells) coated with anti-Human ENA-78
- 20X Wash Buffer (Item B)-RABWASH4: 25 mL of 20X concentrated solution
- Lyophilized Human ENA-78 Protein Standard (Item C)-RAB0130C-1VL: 2 vials of Human ENA-78. 1 vial is enough to run each standard in duplicate
- Biotinylated Human ENA-78 Detection Antibody (Item F)-RAB0130D-1VL: 2 vials of biotinylated anti-Human ENA-78. Each vial is enough to assay half the microplate
- HRP-Streptavidin (Item G)- RABHRP5: 200 µL 500X concentrated HRP-conjugated streptavidin
- ELISA Colorimetric TMB Reagent (HRP Substrate, Item H)- RABTMB3: 12 mL of 3,3,5,5'-tetramethylbenzidine (TMB) in buffer solution
- ELISA Stop Solution (Item I)-RABSTOP3: 8 mL of 0.2 M sulfuric acid
- ELISA 1x Assay/Sample Diluent Buffer A (Item D1)- RABELADA-30mL: 30 mL of diluent buffer, 0.09% sodium azide as preservative
- ELISA 5x Assay/Sample Diluent Buffer B (Item E1)- RABELADB-15mL: 15 mL of 5X concentrated buffer

Reagents and Equipment Required

(Not Provided)

- Microplate reader capable of measuring absorbance at 450 nm
- Precision pipettes to deliver 2 µL to 1 mL volumes
- Adjustable 1-25 mL pipettes for reagent preparation
- 100 mL and 1liter graduated cylinders
- Absorbent Paper
- Distilled or deionized water
- Tubes to prepare standard or sample dilutions
- Log-log graph paper or computer and software for ELISA data analysis

Precautions and Disclaimer

For R&D use only. Not for drug, household, or other uses. Please consult the Safety Data Sheet. For information regarding hazards and safe handling practices.

Preparation Instructions

Reagent Preparation

1. Bring all reagents and samples to room temperature (18-25 °C) before use.
2. Assay Diluent B (Item E) should be diluted 5-fold with deionized or distilled water before use.

Sample Dilution

Assay Diluent A (Item D) should be used for dilution of serum and plasma samples. 1X Assay Diluent B (Item E) should be used for dilution of cell culture supernatant samples. The suggested dilution for normal serum/plasma is 2-10-fold.

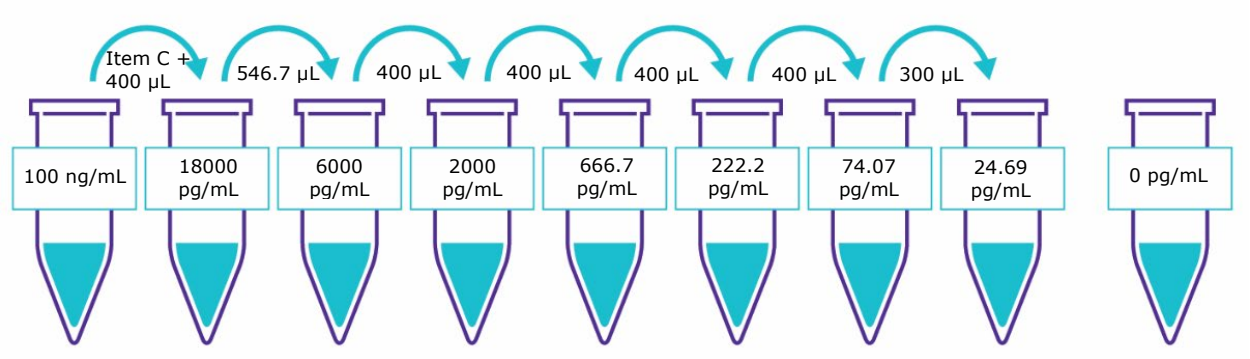
Note: Levels of ENA-78 may vary between different samples. Optimal dilution factors for each sample must be determined by the investigator.

Preparation of standard

1. Briefly spin a vial of Item C.
2. Add 400 µL Assay Diluent A (for serum/plasma samples) or 1X Assay Diluent B (for cell culture medium) into Item C vial to prepare a 100 ng/mL standard.
3. Dissolve the powder thoroughly by a gentle mix.
4. Add 120 µL ENA-78 standard from the vial of Item C, into a tube with 546.7 µL Assay Diluent A or 1X Assay Diluent B to prepare an 18000 pg/mL stock standard solution.
5. Pipette 400 µL Assay Diluent A or 1X Assay Diluent B into each tube. Use the stock standard solution to produce a dilution series (shown below).
6. Mix each tube thoroughly before the next transfer. Assay Diluent A or 1X Assay Diluent B serves as the zero standard (0 pg/mL). B serves as the zero standard (0 pg/mL).
7. If the Wash Concentrate (20X) (Item B) contains visible crystals, warm to room temperature and mix gently until dissolved.
8. Dilute 20 mL of Wash Buffer Concentrate into deionized or distilled water to yield 400 mL of 1X Wash Buffer.
9. Briefly spin the Detection Antibody vial (Item F) before use.
10. Add 100 µL of 1X Assay Diluent B (Item E) into the vial to prepare a detection antibody concentrate.
11. Pipette up and down to mix gently (the concentrate can be stored at 4 °C for 5 days). The detection antibody concentrate should be diluted 80-fold with 1X Assay Diluent B (Item E) and used in step 5 of Part VI Assay Procedure.
12. Briefly spin the HRP-Streptavidin concentrate vial (Item G) and pipette up and down to mix gently before use, as precipitates may form during storage. HRP-Streptavidin concentrate should be diluted 500-fold with 1X Assay Diluent B (Item E).

    For example: Briefly spin the vial (Item G) and pipette up and down to mix gently. Add 20 µL of HRP-Streptavidin concentrate into a tube with 10 mL 1X Assay Diluent B to prepare a final 500-fold diluted HRP-Streptavidin solution (don't store the diluted solution for next day use). Mix well.

	120 µL	200 µL	200 µL	200 µL	200 µL	200 µL	200 µL	200 µL	
		Std 1	**Std 2**	**Std 3**	**Std 4**	**Std 5**	**Std 6**	**Std 7**	**0 Std**
Diluent volume	Item C + 400 µL	546.7 µL	400 µL	400 µL	400 µL	400 µL	400 µL	300 µL	300 µL
Conc.	100 ng/mL	18000 pg/mL	6000 pg/mL	2000 Pg/mL	666.7 pg/mL	222.2 pg/mL	74.07 pg/mL	24.69 pg/mL	0 Pg/mL



Storage/Stability

The entire kit may be stored at -20º C for up to 1 year from the date of shipment. Avoid repeated freeze-thaw cycles. The kit may be stored at 4 °C for up to 6 months. For extended storage, it is recommended to store at -80 °C. For prepared reagent storage, see table below.

Procedure

1. Bring all reagents and samples to room temperature (18–25 °C) before use. It is recommended that all standards and samples be run at least in duplicate.
2. Label removable 8-well strips as appropriate for your experiment.
3. Add 100 µL of each standard (see Reagent Preparation step 3) and sample into appropriate wells. Cover wells and incubate for 2.5 hours at room temperature with gentle shaking.
4. Discard the solution and wash 4 times with 1X Wash Solution. Wash by filling each well with Wash Buffer (300 µL) using a multi-channel Pipette or autowasher. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
5. Add 100 µL of 1X prepared biotinylated antibody (Reagent Preparation step 6) to each well. Incubate for 1 hour at room temperature with gentle shaking.
6. Discard the solution. Repeat the wash as in step 4.
7. Add 100 µL of prepared Streptavidin solution (see Reagent Preparation step 7) to each well. Incubate for 45 minutes at room temperature with gentle shaking.
8. Discard the solution. Repeat the wash as in step 4.
9. Add 100 µL of TMB One-Step Substrate Reagent (Item H) to each well. Incubate for 30 minutes at room temperature in the dark with gentle shaking.
10. Add 50 µL of Stop Solution (Item I) to each well. Read at 450 nm immediately.

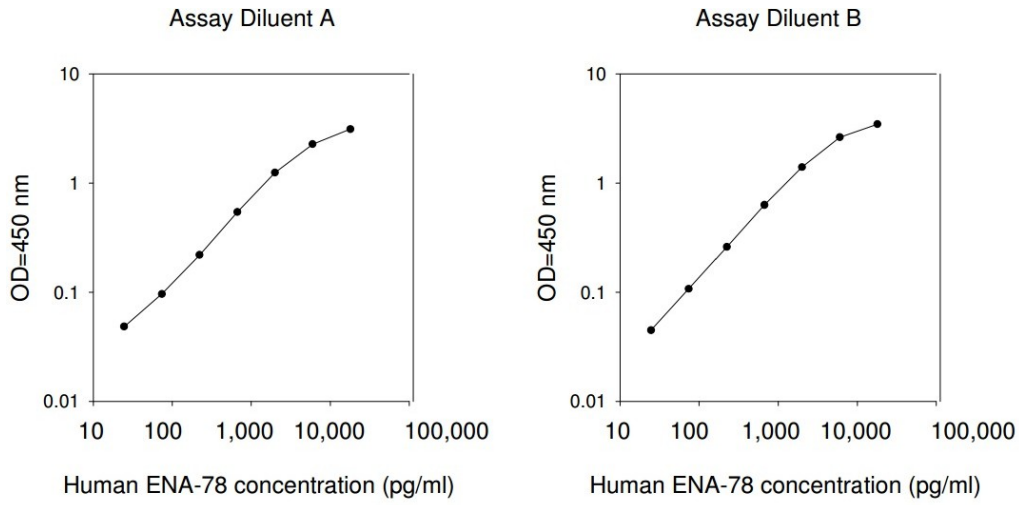
Results

Calculations

Calculate the mean absorbance for each set of duplicate standards, controls and samples, and subtract the average zero standard optical density. Plot the standard curve on log-log graph paper or using Sigma plot software, with standard concentration on the x-axis and absorbance on the y-axis. Draw the best-fit straight line through the standard points.

Typical Data

These standard curves are for demonstration only. A standard curve must be run with each assay.



Sensitivity

The minimum detectable dose of Human ENA-78 was determined to be 10 pg/mL. Minimum detectable dose is defined as the analyte concentration resulting in an absorbance that is 2 standard deviations higher than that of the blank (diluent buffer).

Spiking and Recovery

Recovery was determined by spiking various levels of Mouse Leptin into the sample types listed below. Mean recoveries are as follows:

Sample Type	Average % Recovery	Range (%)
Serum	93.39	82-102
Plasma	92.15	81-101
Cell culture media	102.15	82-106

Linearity

Sample	Type	Serum	Plasma	Cell Culture Media
1:2	Average % of Expected	93	94	94
	Range (%)	82-102	83-102	83-102
1:4	Average % of Expected Range	94	93	96
	Range (%)	83-104	82-102	84-103

Reproducibility

Intra-Assay CV%: <10%

Inter-Assay CV%: <12%.

Specificity

This ELISA kit shows no cross-reactivity with any of the cytokines tested: Human Angiogenin, BDNF, BLC, FGF-4, IL-1 alpha, IL-1 beta, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL- 11, IL-12 p70, IL-12 p40, IL-13, IL-15, I-309, IP-10, G-CSF, GM-CSF, IFN-gamma, Leptin (OB), MCP-1, MCP-3, MDC, MIP-1 alpha, MIP-1 beta, MIP-1 delta, MMP-1, -2, -7, -8, -9, - 10, -12, -13, PARC, RANTES, SCF, TARC, TGF-beta, TIMP-1, TIMP-2, TNF-alpha, TNF- beta, TPO, VEG.

Appendix

Troubleshooting

Problem	Cause	Solution
Poor standard curve	Inaccurate pipetting	Check pipettes
	Improper standard dilution	Briefly centrifuge Item C and dissolve the powder thoroughly by gently mixing.
Low signal	Improper preparation of standard and/or biotinylated antibody	Briefly spin down vials before opening. Dissolve the powder thoroughly.
	Too brief incubation times	Ensure sufficient incubation time. Assay procedure step 3 may be done overnight at 4 °C with gentle shaking (Note: may increase overall signals including background).
	Inadequate reagent volumes or improper dilution	Check pipettes and ensure correct preparation
Large CV	Inaccurate Pipetting	Check Pipettes
	Air Bubbles in well	Remove bubbles in well
High background	Plate is insufficiently washed	Review the manual for proper wash. If using a plate washer, ensure that all ports are unobstructed.
	Contaminated wash buffer	Make fresh wash buffer
Low sensitivity	Improper storage of the ELISA kit	Store the standard at <–70 °C after reconstitution, others at 4 °C. Keep substrate solution protected from light
	Stop solution	Add stop solution to each well before reading plate

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