Erap1 (Mouse) ELISA Kit

Catalog Number KA5865

96 assays

Version: 02

Intended for research use only

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Introduction

Intended Use

For the quantitation of Mouse Erap1 concentrations in cell culture supernatants, serum and plasma (heparin, EDTA).

Background

Type 1 tumor necrosis factor receptor shedding aminopeptidase regulator, also known as endoplasmic reticulum aminopeptidase 1 (ARTS-1), is a protein which in humans is encoded by the ARTS-1 gene. This gene is mapped to 13; 13 C1. Endoplasmic reticulum amino peptidase 1 is active in the endoplasmic reticulum, which is involved in protein processing and transport. This protein is an aminopeptidase, which is an enzyme that cleaves peptides at the N-terminal into smaller fragments called amino acids.

Principle of the Assay

The Erap1 (Mouse) ELISA Kit is a solid-phase immunoassay specially designed to measure Mouse Erap1 with a 96-well strip plate that is pre-coated with antibody specific for Erap1. The detection antibody is a biotinylated antibody specific for Erap1. The capture antibody is a monoclonal antibody from rat and the detection antibody is a biotinylated polyclonal antibody from goat. The kit contains recombinant Mouse Erap1 with immunogen: Expression system for standard: NS0; Immunogen sequence: M1-L930. The kit is analytically validated with ready-to-use reagents.

To measure Mouse Erap1, add standards and samples to the wells, then add the biotinylated detection antibody. Wash the wells with PBS or TBS buffer, and add Avidin-Biotin-Peroxidase Complex (ABC-HRP). Wash away the unbounded ABC-HRP with PBS or TBS buffer and add TMB. TMB is an HRP substrate and will be catalyzed to produce a blue color product, which changes into yellow after adding the acidic stop solution. The absorbance of the yellow product at 450 nm is linearly proportional to Mouse Erap1 in the sample. Read the absorbance of the yellow product in each well using a plate reader, and benchmark the sample wells' readings against the standard curve to determine the concentration of Mouse Erap1 in the sample.

Uniprot ID: Q9EQH2

General Information

Materials Supplied

List of component

Component	Amount	Storage of opened/reconstituted material
Anti-Mouse Erap1 Pre-coated 96-well Strip Microplate	12 strips of 8 wells	Return unused wells to the foil pouch. Reseal along entire edge of zip-seal. May be stored for up to 1 month at 4°C provided this is within the expiration date of the kit.
Mouse Erap1 Standard	20 ng/tube x 2	Discard the Erap1 stock solution after 12 hours at 4°C. May be stored at -20°C for 48 hours.
Mouse Erap1 Biotinylated antibody (100x)	100 µL	May be stored for up to 1 month at 4°C provided this is within the expiration date of the kit.
Avidin-Biotin-Peroxidase Complex (100x)	100 µL	
Sample Diluent	30 mL	
Antibody Diluent	12 mL	
Avidin-Biotin-Peroxidase Diluent	12 mL	
Color Developing Reagent (TMB)	10 mL	
Stop Solution	10 mL	
Wash Buffer (25x)	20 mL	
Plate Sealers	4 slides	

Storage Instruction

Store at -20°C for one year. For short term storage and frequent use, store at 4°C for up to one month. Avoid repeated freeze-thaw cycles.

Materials Required but Not Supplied

- ✓ Microplate Reader capable of reading absorbance at 450 nm.
- ✓ Automated plate washer (optional).
- ✓ Pipettes and pipette tips capable of precisely dispensing 0.5 µL through 1 mL volumes of aqueous solutions.
- ✓ Multichannel pipettes are recommended for large amount of samples.
- ✓ Deionized or distilled water.
- ✓ 500 mL graduated cylinders.
- ✓ Test tubes for dilution.

Precautions for Use

This protocol must be read in its entirety before using this product. For research use only. Not for use in diagnostic procedures.

✓ Preparations Before Assay

Please read the following instructions before starting the experiment.

1. Read this manual in its entirety in order to minimize the chance of error.
2. Confirm that you have the appropriate non-supplied equipment available.
3. Confirm that the species, target antigen, and sensitivity of this kit are appropriate for your intended application.
4. Confirm that your samples have been prepared appropriately based upon recommendations (see Sample Preparation) and that you have sufficient sample volume for use in the assay.
5. When first using a kit, appropriate validation steps should be taken before using valuable samples. Confirm that the kit adequately detects the target antigen in your intended sample type(s) by running control samples.
6. If the concentration of target antigen within your samples is unknown, a preliminary experiment should be run using a control sample to determine the optimal sample dilution (see Sample Preparation).
7. To inspect the validity of experiment operation and the appropriateness of sample dilution proportion, pilot experiment using standards and a small number of samples is recommended.
8. Before using the Kit, spin tubes and bring down all components to the bottom of tubes.
9. Don’t let 96-well plate dry, for dry plate will inactivate active components on plate.
10. Don’t reuse tips and tubes to avoid cross contamination.
11. Avoid using the reagents from different batches together.
12. The kit should not be used beyond the expiration date on the kit label. Any variation in diluent, operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding. Variations in sample collection, processing, and storage may cause sample value differences.

Assay Protocol

Reagent Preparation

- ✓ Bring all reagents to room temperature (18-25°C) prior to use. Please DO NOT equilibrate unused plate well strips to room temperature. They should be sealed and stored in the original packaging. The assay can also be done at room temperature however we recommend doing it at 37°C for best consistency with our QC results. Also the TMB incubation time estimate (15-25 min) is based on 37°C.
  - Wash buffer

    Prepare 500 mL of Working Wash Buffer by diluting the supplied 20 mL of Wash Buffer (25 x) with 480 mL of deionized or distilled water. If crystals have formed in the concentrate, warm to room temperature and mix it gently until crystals have completely dissolved.
  - Biotinylated Anti-Mouse Erap1 antibody

    It is recommended to prepare this reagent immediately prior to use by diluting the Mouse Erap1 Biotinylated antibody (100x) 1:100 with Antibody Diluent. Prepare 100 µL by adding 1 µL of Biotinylated antibody (100x) to 99 µL of Antibody Diluent for each well. Mix gently and thoroughly and use within 2 hours of generation.
  - Avidin-Biotin-Peroxidase Complex

    It is recommended to prepare this reagent immediately prior to use by diluting the Avidin-Biotin-Peroxidase Complex (100x) 1:100 with Avidin-Biotin-Peroxidase Diluent. Prepare 100 µL by adding 1 µL of Avidin-Biotin-Peroxidase Complex (100x) to 99 µL of Avidin-Biotin-Peroxidase Diluent for each well. Mix gently and thoroughly and use within 2 hours of generation.
  - Mouse Erap1 Standard

    It is recommended that the standards be prepared no more than 2 hours prior to performing the experiment. Use one 20 ng of lyophilized Mouse Erap1 standard for each experiment. Gently spin the vial prior to use. Reconstitute the standard to a stock concentration of 20 ng/mL using 1mL of sample diluent. Allow the standard to sit for a minimum of 10 minutes with gentle agitation prior to making dilutions.
  - Microplate

    The included microplate is coated with capture antibodies and ready-to-use. It does not require additional washing or blocking. The unused well strips should be sealed and stored in the original packaging.
- ✓ Dilution of Mouse Erap1 Standard
  1. Number tubes 1-8. Final Concentrations to be Tube # 1 –20,000.00 pg/mL, # 2 –10,000.00 pg/mL, # 3 – 5,000.00 pg/mL, # 4 – 2,500.00 pg/mL, # 5 – 1,250.00 pg/mL, # 6 – 625.00 pg/mL, # 7 – 312.50 pg/mL, #8 –Sample Diluent serves as the zero standard (0 pg/mL).
  2. For standard #1, add 1000 µL of undiluted standard stock solution to tube #1.
  3. Add 300 µL of sample diluent to tubes # 2-7.
  4. To generate standard #2, add 300 µL of standard #1 from tube #1 to tube #2 for a final volume of 600

µL. Mix thoroughly.

5. To generate standard #3, add 300 µL of standard #2 from tube #2 to tube #3 for a final volume of 600 µL. Mix thoroughly.
6. Continue the serial dilution for tube #4-7.

Sample Preparation

✓ Sample Preparation and Storage

These sample collection instructions and storage conditions are intended as a general guideline and the sample stability has not been evaluated.

- Cell culture supernatants: Clear sample of particulates by centrifugation, assay immediately or store samples at -20°C.
- Serum: Use a serum separator tube (SST) and allow serum to clot at room temperature for about four hours. Then, centrifuge for 15 min at approximately 1,000 x g. assay immediately or store samples at -20°C.
- Plasma: Collect plasma using heparin or EDTA as an anticoagulant. Centrifuge for 15 min at approximately 1,000 x g. Assay immediately or store samples at -20°C.

  **Note: it is important to not use anticoagulants other than the ones described above to treat plasma for other anticoagulants could block the antibody binding site.*

✓ *Sample Collection Notes*

1. *We recommend that samples are used immediately upon preparation.*
2. *Avoid repeated freeze/thaw cycles for all samples.*
3. *In the event that a sample type not listed above is intended to be used with the kit, it is recommended that the customer conduct validation experiments in order to be confident in the results.*
4. *Due to chemical interference, the use of tissue or cell extraction samples prepared by chemical lysis buffers may result in inaccurate results.*
5. *Due to factors including cell viability, cell number, or sampling time, samples from cell culture supernatant may not be detected by the kit.*
6. *Samples should be brought to room temperature (18-25°C) before performing the assay without the use of extra heating.*
7. *Sample concentrations should be predicted before being used in the assay. If the sample concentration is not within the range of the standard curve, users must determine the optimal sample dilutions for their particular experiments.*
8. *Abnova is responsible for the quality and performance of the kit components but is NOT responsible for the performance of customer supplied samples used with the kit.*

✓ Sample Dilution Guideline

The user needs to estimate the concentration of the target protein in the sample and use an appropriate

dilution factor so that the diluted target protein concentration falls in the range of O.D. values of the standard curve. Dilute the sample using provided diluent buffer. Pilot tests using a dilution series of each sample type is necessary. The sample must be mixed thoroughly with Sample Diluent.

Assay Procedure

It is recommended that all reagents and materials be equilibrated to room temperature (18-25°C) prior to the experiment (see Reagent Preparation if you have missed this information).

1. Prepare all reagents and working standards as directed previously.
2. Remove excess microplate strips from the plate frame and seal and store them in the original packaging.
3. Add 100 µL of the standard, samples, or control per well. Add 100 µL of the Sample Diluent into the zero well. At least two replicates of each standard, sample, or control is recommended.
4. Cover with the plate sealer provided and incubate for 120 minutes at room temperature (or 90 minutes at 37°C).
5. Remove the cover and discard the liquid in the wells into an appropriate waste receptacle. Invert the plate on the benchtop onto a paper towel and tap the plate to gently blot any remaining liquid. It is recommended that the wells are not allowed to completely dry at any time.
6. Add 100 µL of the prepared 1x Biotinylated Anti-Mouse Erap1 antibody to each well.
7. Cover with plate sealer and incubate for 90 minutes at room temperature (or 60 minutes at 37°C).
8. Wash the plate 3 times with the 1x wash buffer.

   a. Discard the liquid in the wells into an appropriate waste receptacle. Then, invert the plate on the benchtop onto a paper towel and tap the plate to gently blot any remaining liquid. It is recommended that the wells are not allowed to completely dry at any time.

   b. Add 300 µL of the 1x wash buffer to each assay well. (For cleaner background incubate for 60 seconds between each wash).

   c. Repeat steps a-b 2 additional times.

   d. Discard the wash buffer in the wells into an appropriate waste receptacle. Then, invert the plate on the benchtop onto a paper towel and tap the plate to gently blot any remaining liquid.
9. Add 100 µL of the prepared 1x Avidin-Biotin-Peroxidase Complex into each well. Cover with the plate sealer provided and incubate for 40 minutes at RT (or 30 minutes at 37°C).
10. Wash the plate 5 times with the 1x wash buffer.

    a. Discard the liquid in the wells into an appropriate waste receptacle. Then, invert the plate on the benchtop onto a paper towel and tap the plate to gently blot any remaining liquid. It is recommended that the wells are not allowed to completely dry at any time.

    b. Add 300 µL of the 1x wash buffer to each assay well. (For cleaner background incubate for 60 seconds between each wash).

    c. Repeat steps a-b 4 additional times.

    d. Discard the wash buffer in the wells into an appropriate waste receptacle. Then, invert the plate on the benchtop onto a paper towel and tap the plate to gently blot any remaining liquid.

11. Add 90 µL of Color Developing Reagent to each well. Cover with the plate sealer provided and incubate in the dark for 30 minutes at RT (or 15-25 minutes at 37°C). (The optimal incubation time must be empirically determined. A guideline to look for is blue shading the top four standard wells, while the remaining standards remain clear.).
12. Add 100 µL of Stop Solution to each well. The color should immediately change to yellow.
13. Within 30 minutes of stopping the reaction, the O.D. absorbance should be read with a microplate reader at 450 nm.

✓ *Assay protocol Notes*

1. *Solutions: To avoid cross-contamination, change pipette tips between additions of each standard, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.*
2. *Applying Solutions: All solutions should be added to the bottom of the ELISA plate well. Avoid touching the inside wall of the well. Avoid foaming when possible.*
3. *Assay Timing: The interval between adding sample to the first and last wells should be minimized. Delays will increase the incubation time differential between wells, which will significantly affect the experimental accuracy and repeatability. For each step in the procedure, total dispensing time for addition of reagents or samples should not exceed 10 minutes.*
4. *Incubation: To prevent evaporation and ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary. Do not allow wells to sit uncovered for extended periods of time between incubation steps. Do not let wells dry out at any time during the assay. Strictly observe the recommended incubation times and temperatures.*
5. *Washing: Proper washing procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings. Residual liquid in the reaction wells should be patted dry against absorbent paper during the washing process. Do not put absorbent paper directly into the reaction wells.*
6. *Controlling Substrate Reaction Time: After the addition of the TMB Substrate, periodically monitor the color development. Stop color development before the color becomes too deep by adding Stop Solution. Excessively strong color will result in inaccurate absorbance readings.*
7. *Reading: The microplate reader should be preheated and programmed prior to use. Prior to taking OD readings, remove any residual liquid or fingerprints from the underside of the plate and confirm that there are no bubbles in the wells.*
8. *Reaction Time Control: Control reaction time should be strictly followed as outlined.*
9. *Stop Solution: The Stop Solution contains an acid, therefore proper precautions should be taken during its use, such as protection of the eyes, hands, face, and clothing.*
10. *To minimize external influence on the assay performance, operational procedures and lab conditions (such as room temperature, humidity, incubator temperature) should be strictly controlled. It is also strongly suggested that the whole assay is performed by the same operator from the beginning to the end.*

Data Analysis

Calculation of Results

Average the duplicate readings for each standard, sample, and control. Subtract the average zero standard O.D. reading.

It is recommended that a standard curve be created using computer software to generate a four parameter logistic (4-PL) curve-fit. A free program capable of generating a four parameter logistic (4-PL) curve-fit can be found online at: www.myassays.com/four-parameter-logisticcurve.assay.

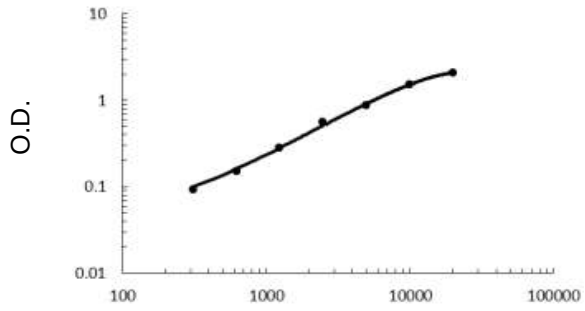
Alternatively, plot the mean absorbance for each standard against the concentration. The measured concentration in the sample can be interpolated by using linear regression of each average relative OD against the standard curve generated using curve fitting software. This will generate an adequate but less precise fit of the data.

For diluted samples, the concentration reading from the standard curve must be multiplied by the dilution factor.

✓ Erap1 (Mouse) ELISA Kit Standard Curve Sample

The highest O.D. value might be higher or lower than in the example. The experiment result is statistically significant if the highest O.D. value is no less than 1.0.

Concentration (pg/mL)	0	312	625	1250	2500	5000	10000	20000
O.D	0.007	0.093	0.152	0.281	0.557	0.882	1.535	2.081



Concentration (pg/mL)

A standard curve is provided for demonstration only. A standard curve should be generated for each set of samples assayed.

Performance Characteristics

✓ Detection Range: 312g/mL- 20000 pg/mL

✓ Sensitivity: < 50 pg/mL

* The sensitivity or the minimum detectable dose (MDD) is the lower limit of target protein that can be

detected by the kit. It is determined by adding two standard deviations to the mean O.D. value of twenty (20) blank wells and calculating the corresponding concentration.

✓ Specificity: Natural and recombinant Mouse Erap1

✓ Cross-reactivity: This kit is for the detection of Mouse Erap1. No Significant cross-reactivity or interference between Erap1 and analogs was observed. This claim is limited by existing techniques therefore cross-reactivity may exist with untested analogs.

✓ Intra/Inter Assay Variability

- Intra-Assay Precision (Precision within an assay)

  Three samples of known concentration were tested on one plate to assess intra-assay precision.

- Inter-Assay Precision (Precision across assays)

  Three samples of known concentration were tested in separate assays to assess inter-assay precision.

	Intra-Assay Precision			Inter-Assay Precision		
Sample	1	2	3	1	2	3
n	16	16	16	24	24	24
Mean (pg/mL)	505	1975	8963	2058	587	9289
Standard deviation	29.29	118.50	412.30	125.54	40.50	678.10
CV (%)	5.8%	6.0%	4.6%	6.1%	6.9%	7.3%

✓ Reproducibility

To assay reproducibility, three samples with differing target protein concentrations were assayed using four different lots.

Lots	Lot1 (pg/mL)	Lot2 (pg/mL)	Lot3 (pg/mL)	Lot4 (pg/mL)	Mean (pg/mL)	Standard Deviation	CV (%)
Sample 1	505	578	524	604	552	39.9	7.2%
Sample 2	1975	2096	1918	1952	1985	67.07	3.3%
Sample 3	8965	10445	9390	9719	9629	541.90	5.6%

*number of samples for each test n=16.

Resources

Plate Layout

12								
11								
10								
9								
8								
7								
6								
5								
4								
3								
2								
1								
	A	B	C	D	E	F	G	H