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# **PARN ELISA Kit**





### Overview

| Quantity:                | 96 tests        |
|--------------------------|-----------------|
| Target:                  | PARN            |
| Reactivity:              | Mouse           |
| Method Type:             | Sandwich ELISA  |
| Detection Range:         | 35-10.000 pg/mL |
| Minimum Detection Limit: | 35 pg/mL        |
| Application:             | ELISA           |

| Product Details    |   |
|--------------------|---|
| Purpose:           | Mouse DAN ELISA Kit for cell culture supernatants, plasma, and serum samples.   |
| Sample Type:       | Serum, Plasma, Cell Culture Supernatant   |
| Analytical Method: | Quantitative  |
| Detection Method:  | Colorimetric  |
| Specificity:       | This ELISA antibody pair detects mouse DAN, no cross-reactivity with rhDAN, rhCOCO, rmBMP-4 or rmGremlin  |
| Characteristics:   | <ul> <li>Strip plates and additional reagents allow for use in multiple experiments</li> <li>Quantitative protein detection</li> <li>Establishes normal range</li> <li>The best products for confirmation of antibody array data</li> </ul> |
| Components:        | <ul><li>Pre-Coated 96-well Strip Microplate</li><li>Wash Buffer</li></ul>   |

## **Product Details**

- Stop Solution
- Assay Diluent(s)
- · Lyophilized Standard
- Biotinylated Detection Antibody
- · Streptavidin-Conjugated HRP
- TMB One-Step Substrate

#### Material not included:

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

# Target Details

| Target:           | PARN                |
|-------------------|---------------------|
| Alternative Name: | DAN (PARN Products) |
| Gene ID:          | 74108               |
| UniProt:          | Q8VDG3              |

| Application Details |   |
|---------------------|---|
| Application Notes:  | Recommended Dilution for serum and plasma samples2 fold                     |
| Sample Volume:      | 100 μL  |
| Plate:              | Pre-coated  |
| Protocol:           | 1. Prepare all reagents, samples and standards as instructed in the manual. |
|                     | 2. Add 100 µL of standard or sample to each well.                           |
|                     | 3. Incubate 2.5 h at RT or O/N at 4 °C.                                     |
|                     | 4. Add 100 μL of prepared biotin antibody to each well.                     |
|                     | 5. Incubate 1 h at RT.  |
|                     | 6. Add 100 μL of prepared Streptavidin solution to each well.               |
|                     | 7. Incubate 45 min at RT.   |
|                     | 8. Add 100 μL of TMB One-Step Substrate Reagent to each well.               |
|                     | 9. Incubate 30 min at RT.   |
|                     | 10. Add 50 μL of Stop Solution to each well.                                |
|                     | 11. Read at 450 nm immediately.   |

Reagent Preparation:

1. Bring all reagents and samples to room temperature (18 - 25 °C) before use. 2. Sample dilution: If your samples need to be diluted, Assay Diluent C (Item L) should be used for dilution of serum/plasma/culture supernatants. Suggested dilution for normal serum/plasma: 2 fold\*. \* Please note that levels of the target protein may vary between The Mouse DAN ELISA Kit Protocol 3 different specimens. Optimal dilution factors for each sample must be determined by the investigator. 3. Assay Diluent B (Item E) should be diluted 5-fold with deionized or distilled water before use. 4. Preparation of standard: Briefly spin the vial of Item C. Add 400 µL Assay Diluent C (Item L) into Item C vial to prepare a 50 ng/ml standard solution. Dissolve the powder thoroughly by a gentle mix. Add 50 µL DAN standard from the vial of Item C, into a tube with 450 µL Assay Diluent C to prepare a 10,000 pg/ml standard solution. Pipette 300myl Assay Diluent C into each tube. Use the 10,000 pg/ml standard solution to produce a dilution series (shown below). Mix each tube thoroughly before the next transfer. Assay Diluent C serves as the zero standard (0 pg/ml). 200 µL 50 µL Item C + 450 200myl 200 µL 200 µL 200 µL 200 µL 10,000 4,000 1,600 640 256 102.4 40.96 0 pg/ml pg/ml pg/ml pg/ml pg/ml pg/ml pg/ml pg/ml pg/ml 5. If the Wash Concentrate (20x) (Item B) contains visible crystals, warm to room temperature and mix gently until dissolved. Dilute 20 mL of Wash Buffer Concentrate into deionized or distilled water to yield 400 mL of 1x Wash Buffer. 6. Briefly spin the Detection Antibody vial (Item F) before use. Add 100 µL of 1x Assay Diluent B (Item E) into the vial to prepare a detection antibody concentrate. Pipette up and down to mix gently The Mouse DAN ELISA Kit Protocol 4 (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 and used in step 4 of Part VI Assay Procedure. 7. Briefly spin the HRP-Streptavidin concentrate vial (Item G) and pipette up and down to mix gently before use. HRP-Streptavidin concentrate should be diluted 250-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 40  $\mu$ L of HRP-Streptavidin concentrate into a tube with 10 mL 1x Assay Diluent B to prepare a 250-fold diluted HRP-Streptavidin solution (don't store the diluted solution for next day use). Mix well.

Assay 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. Add 100  $\mu$ L of each standard (see Reagent Preparation step 2) and sample into appropriate wells. Cover well and incubate for 2.5 hours at room temperature or over night at 4 °C with gentle shaking. 3. Discard 4. Add 100  $\mu$ L of 1x prepared biotinylated antibody (Reagent Preparation step 6) to each well. Incubate for 1 hour at room temperature with gentle shaking. 5. Discard the solution. Repeat the wash as in step 3. 6. Add 100  $\mu$ L of prepared Streptavidin solution (see Reagent Preparation step 7) to each well. Incubate for 45 minutes at room temperature with gentle

| shaking. 7. Discard the solution. Repeat the wash as in step 3. 8. Add 100 $\mu L$ of TMB One-Step $\mu L$ | ) |
|--|---|
| Substrate Reagent (Item H) to each well. Incubate for 30 minutes at room temperature in the                |   |
| dark with gentle shaking. 9. Add 50 $\mu L$ of Stop Solution (Item I) to each well. Read at 450 nm         |   |
| immediately.   |   |

Calculation of Results:

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.

Assay Precision: Intra-Assay: CV<10%

Inter-Assay: CV<12%

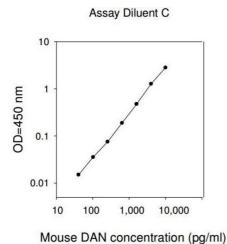
Restrictions: For Research Use only

# Handling

| Handling Advice: | Avoid repeated freeze-thaw cycles.   |
|------------------|--|
| Storage:         | -20 °C   |
| Storage Comment: | The entire kit may be stored at -20°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. |

# **Images**

Expiry Date:



6 months

#### **ELISA**

Image 1.