

ELISA Kit for Measuring Human DJ-1/PARK7

CircuLex Human DJ-1/PARK7 ELISA Kit Ver.2

Cat#: CY-9050V2

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Intended Use

The MBL Research Product **CircuLex Human DJ-1/PARK7 ELISA Kit Ver.2** is used for the quantitative and highly sensitive measurement of human DJ-1/PARK7 in serum and cell lysate samples.

Individual users should determine appropriate conditions when using other types of samples.

This assay kit is for research use only and not for use in diagnostic or therapeutic procedures.

Storage

- Upon receipt store all components at 4°C.
- Don't expose reagents to excessive light.

Introduction

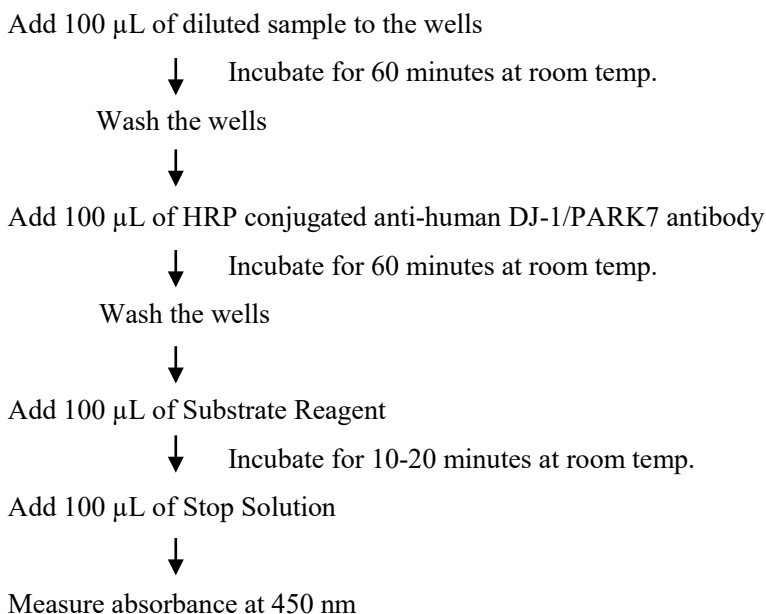
DJ-1 (PARK7/CAP1/RS) was originally cloned as a putative oncogene capable of transforming NIH-3T3 cells in cooperation with H-ras (1), a protein expressed in sperm (2), and a regulator of RNA-protein interactions (3). DJ-1 has also been isolated as a gene associated with autosomal early-onset Parkinson's disease (PD) (4). Taken together, DJ-1 appears to be involved in diverse biological processes (5). First, several lines of evidence suggest that DJ-1 plays a role in the oxidative stress response (6, 7). In cultured mammalian cells, DJ-1 quenches reactive oxygen species and is converted into a variant with a more acidic isoelectric point (6, 8). Therefore, DJ-1 protects against reactive oxygen species-induced cell death, and its suppression with small interfering RNA (siRNA) sensitizes cells to such insults (7-10). Second, DJ-1 modulates transcription through interaction with DJ-1-binding protein (11) as well as with protein inhibitor of activated STAT (PIAS) (12). The latter modulates the activity of various transcription factors, Third, DJ-1 has been recognized as a regulatory subunit of an RNA-binding protein (13), Fourth, DJ-1, which is structurally related to the molecular chaperone Hsp31, may have chaperone activity itself, preventing heat-induced aggregation of substrate proteins, including alpha-synuclein (14).

In addition, several lines of evidence suggest that DJ-1 plays a role in human tumorigenesis. First, breast cancer patients have elevated levels of circulating DJ-1 and anti-DJ-1 autoantibodies compared to healthy and non-breast cancer patients (15). Secondly, DJ-1 protein is increased in primary non-small cell lung carcinoma samples (16). Thirdly, treatment of cells from the human lung cancer cell line NCI-H157 with paclitaxel and MEK inhibitor U0126 leads to a decrease in DJ-1 protein expression (16).

Principle of the Assay

The CircuLex Human DJ-1/PARK7 ELISA Kit Ver.2 employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody (2H2) specific for human DJ-1 has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any DJ-1 present is bound by the immobilized antibody. After washing away any unbound substances, an HRP conjugated antibody specific for DJ-1 is added to the wells. Following a wash to remove any unbound antibody HRP conjugate, the remaining conjugate is allowed to react with the substrate H₂O₂-tetramethylbenzidine. The reaction is stopped by addition of acidic solution and absorbance of the resulting yellow product is measured at 450 nm. The absorbance is proportional to the concentration of DJ-1. A standard curve is constructed by plotting absorbance values versus DJ-1 concentrations of calibrators, and concentrations of unknown samples are determined using this standard curve.

Summary of Procedure



Materials Provided

All samples and standards should be assayed in duplicate. The following components are supplied and are sufficient for the one 96-well microplate kit.

Microplate: One microplate supplied ready to use, with 96 wells (12 strips of 8-wells) in a foil, zip-lock bag with a desiccant pack. Wells are coated with anti-human DJ-1/PARK7 monoclonal antibody (2H2) as a capture antibody.

10X Wash Buffer: One 100 mL bottle of 10X buffer containing Tween®-20

Dilution Buffer: One bottle containing 50 mL of 1X buffer; use for sample dilution. Ready to use.

Human DJ-1/PARK7 Standard: One vial containing X* ng of lyophilized human DJ-1/PARK7
***The amount is changed depending on lot. See the real "User's Manual" included in the kit box.**

20X HRP conjugated Detection Antibody: One vial containing 0.6 mL of HRP (horseradish peroxidase) conjugated anti-human DJ-1/PARK7 antibody.

Conjugate Dilution Buffer: One bottle containing 12 mL of Conjugate Dilution Buffer.

Substrate Reagent: One bottle containing 20 mL of the chromogenic substrate, tetra methylbenzidine (TMB). Ready to use.

Stop Solution: One bottle supplied ready to use, containing 20 mL of 1 N H₂SO₄.

Materials Required but not Provided

- **Pipettors:** 2-20 μ L, 20-200 μ L and 200-1,000 μ L precision pipettors with disposable tips.
- **Precision repeating pipettor**
- **Orbital microplate shaker**
- **Microcentrifuge and tubes** for sample preparation.
- **Vortex mixer**
- **(Optional) Microplate washer:** Manual washing is possible but not preferable.
- **Plate reader** capable of measuring absorbance in 96-well plates at dual wavelengths of 450 nm/540 nm. Dual wavelengths of 450/550 or 450/595 nm can also be used. The plate can also be read at a single wavelength of 450 nm, which will give a somewhat higher reading.
- **(Optional) Software package facilitating data generation and analysis**
- **500 or 1,000 mL graduated cylinder.**
- **Reagent reservoirs**
- **Deionized water of the highest quality**
- **Disposable paper towels**

Precautions and Recommendations

- **Although we suggest to conduct experiments as outlined below, the optimal experimental conditions will vary depending on the parameters being investigated, and must be determined by the individual user.**
- Allow all the components to come to room temperature before use.
- All microplate strips that are not immediately required should be returned to the zip-lock pouch, which must be carefully resealed to avoid moisture absorption.
- Do not use kit components beyond the indicated kit expiration date.
- Use only the microtiter wells provided with the kit.
- Rinse all detergent residue from glassware.
- Use deionized water of the highest quality.
- Do not mix reagents from different kits.
- The buffers and reagents in this kit may contain preservatives or other chemicals. Care should be taken to avoid direct contact with these reagents.
- Do not mouth pipette or ingest any of the reagents.
- Do not smoke, eat, or drink when performing the assay or in areas where samples or reagents are handled.
- Dispose of tetra-methylbenzidine (TMB) containing solutions in compliance with local regulations.
- Avoid contact with the acidic Stop Solution and Substrate Solution, which contains hydrogen peroxide.
- Wear gloves and eye protection when handling immunodiagnostic materials and samples of human origin, and these reagents. In case of contact with the Stop Solution and the Substrate Solution, wash skin thoroughly with water and seek medical attention, when necessary.
- **Biological samples may be contaminated with infectious agents. Do not ingest, expose to open wounds or breathe aerosols. Wear protective gloves and dispose of biological samples properly.**
- **CAUTION: Sulfuric Acid is a strong acid. Wear disposable gloves and eye protection when handling Stop Solution.**

Sample Collection and Storage

Serum: Use a serum separator tube and allow samples to clot for 60 ± 30 minutes. Centrifuge the samples at 4°C for 10 minutes at 1,000 x g. Remove serum and assay immediately or store samples on ice for up to 6 hours before assaying. Aliquots of serum may also be stored at below -70°C for extended periods of time. Avoid repeated freeze-thaw cycles.

Cell lysate: Prepare cell lysates. Assay immediately or store the samples on ice for a few hours before assaying. Aliquots of the samples may also be stored at below -70°C for extended periods of time. Avoid repeated freeze-thaw cycles.

Other biological samples: MBL has not tested.

(e.g. Remove any particulates by centrifugation and assay immediately or aliquot and store samples at below -70°C. Avoid repeated freeze-thaw cycles. Individual users should determine appropriate conditions when using other types of samples.)

Detailed Protocol

The MBL Research Product **CircuLex Human DJ-1/PARK7 ELISA Kit Ver.2** is provided with removable strips of wells so the assay can be carried out on separate occasions using only the number of strips required for the particular determination. Since experimental conditions may vary, an aliquot of the Human DJ-1/PARK7 Standard within the kit, should be included in each assay as a calibrator. Disposable pipette tips and reagent troughs should be used for all liquid transfers to avoid cross-contamination of reagents or samples.

Preparation of Working Solutions

All reagents need to be brought to room temperature prior to the assay. Assay reagents are supplied ready-to-use, with the exception of **10X Wash Buffer**, **20X HRP-conjugated Detection Antibody** and **Human DJ-1/PARK7 Standard**.

1. Prepare a working solution of Wash Buffer by adding 100 mL of the **10X Wash Buffer** to 900 mL of deionized (distilled) water (ddH₂O). Mix well. Store at 4°C for two weeks or -20°C for long-term storage.
2. Prepare HRP conjugated Detection Antibody by **20-fold** diluting the **20X HRP-conjugated Detection Antibody** with **Conjugate Dilution Buffer** at the time of assay.
Prepare appropriate volume for your assay. Discard any unused HRP-conjugated Detection Antibody after diluted.
3. Reconstitute **Human DJ-1/PARK7 Standard** with **X* mL** of **ddH₂O**. The concentration of the human DJ-1/PARK7 in vial should be **688 ng/mL**, which is referred as a **Master Standard** of human DJ-1/PARK7.

***The amount is changed depending on lot. See the real "User's Manual" included in the kit box.**

Prepare Standard Solutions as follows:

Use the **Master Standard** to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The 1,600 pg/mL standard (Std.1) serves as the highest standard. The **Dilution Buffer** serves as the zero standard (Blank).

| | Volume of Standard | Dilution Buffer | Concentration |
|-------|--------------------------------------|-----------------|---------------|
| Std.0 | 30 µL of Master Standard (688 ng/ml) | 615 µL | 32 ng/mL |
| Std.1 | 30 µL of Master Standard (32 ng/ml) | 570 µL | 1,600 pg/mL |
| Std.2 | 300 µL of Std. 1 (1,600 pg/mL) | 300 µL | 800 pg/mL |
| Std.3 | 300 µL of Std. 2 (800 pg/mL) | 300 µL | 400 pg/mL |
| Std.4 | 300 µL of Std. 3 (400 pg/mL) | 300 µL | 200 pg/mL |
| Std.5 | 300 µL of Std. 4 (200 pg/mL) | 300 µL | 100 pg/mL |
| Std.6 | 300 µL of Std. 5 (100 pg/mL) | 300 µL | 50 pg/mL |
| Std.7 | 300 µL of Std. 6 (50 pg/mL) | 300 µL | 25 pg/mL |
| Blank | - | 300 µL | 0 pg/mL |

Note: Do not use a Repeating pipette. Change tips for every dilution. Wet tip with Dilution Buffer before dispensing. Unused portions of Standards should be aliquoted and stored at below -70°C immediately. Avoid multiple freeze and thaw cycles.

Sample Preparation

Dilute samples with **Dilution Buffer**.

- Serum samples may require 100-fold or less dilution.

e.g. 5 μ L of sample + 495 μ L of Dilution Buffer

Note: Hemolysis may affect the measurement of DJ-1/PARK7 concentrations in serum.

(See "Fig.1" in the section "Example of Test Results" below)

- Cell lysates samples might require 20- to 1,000-fold dilution.

Note: Higher dilution of samples is recommended.

Assay Procedure

1. Remove the appropriate number of microtiter wells from the foil pouch and place them into the well holder. Return any unused wells to the foil pouch, refold, seal with tape and store at 4°C.
2. Dilute samples with **Dilution Buffer**. (See "Sample Preparation" above.)
3. Pipette **100 μ L** of **Standard Solutions (Std1-Std7, Blank)** and **diluted samples** in duplicates, into the appropriate wells.
4. Incubate the plate **at room temperature (ca.25°C) for 60 minutes** shaking at ca. 300 rpm on an orbital microplate shaker.
5. Wash 4-times by filling each well with Wash Buffer (350 μ L) using a squirt bottle, multi-channel pipette, manifold dispenser or microplate washer.
6. Add **100 μ L** of **HRP conjugated Detection Antibody** into each well.
7. Incubate the plate **at room temperature (ca.25°C) for 60 minutes**, shaking at ca. 300 rpm on an orbital microplate shaker.
8. Wash 4-times by filling each well with Wash Buffer (350 μ L) using a squirt bottle, multi-channel pipette, manifold dispenser or microplate washer.
9. Add **100 μ L** of **Substrate Reagent**. Avoid exposing the microtiter plate to direct sunlight. Covering the plate with e.g. aluminum foil is recommended. Return Substrate Reagent to 4°C immediately after the necessary volume is removed
10. Incubate the plate **at room temperature (ca.25°C) for 10-20 minutes**, shaking at ca. 300 rpm on an orbital microplate shaker. The incubation time may be extended up to 30 minutes if the reaction temperature is below than 20°C.
11. Add **100 μ L** of **Stop Solution** to each well in the same order as the previously added Substrate Reagent.
12. Measure absorbance in each well using a spectrophotometric microplate reader at dual wavelengths of 450/540 nm. Dual wavelengths of 450/550 or 450/595 nm can also be used. Read the microplate at 450 nm if only a single wavelength can be used. Wells must be read within 30 minutes of adding the Stop Solution.

Note-1: 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.

Note-2: Reliable standard curves are obtained when either O.D. values do not exceed 0.25 units for the blank (zero concentrations), or 3.0 units for the highest standard concentration.

Note-3: If the microplate reader is not capable of reading absorbance greater than the absorbance of the highest standard, perform a second reading at 405 nm. A new standard curve, constructed using the values measured at 405 nm, is used to determine DJ-1/PARK7 concentration of off-scale samples. The readings at 405 nm should not replace the on-scale readings at 450 nm.

Calculations

Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density. Plot the optical density for the standards versus the concentration of the standards and draw the best curve. The data can be linearized by using log/log paper and regression analysis may be applied to the log transformation. To determine the DJ-1/PARK7 concentration of each sample, first find the absorbance value on the y-axis and extend a horizontal line to the standard curve. At the point of intersection, extend a vertical line to the x-axis and read the corresponding DJ-1/PARK7 concentration. If the samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

1. The dose-response curve of this assay fits best to a sigmoidal 5-parameter logistic equation. The results of unknown samples can be calculated with any computer program having a 5-parameter logistic function. It is important to make an appropriate mathematical adjustment to accommodate for the dilution factor.
2. Most microtiter plate readers perform automatic calculations of analyte concentration. The calibration curve is constructed by plotting the absorbance (Y) of calibrators versus log of the known concentration (X) of calibrators, using the four-parameter function. Alternatively, the logit log function can be used to linearize the calibration curve (i.e. logit of absorbance (Y) is plotted versus log of the known concentration (X) of calibrators).

Measurement Range

The measurement range is 25 pg/mL to 1,600 pg/mL. Any sample reading higher than the highest standard should be diluted with Dilution Buffer in higher dilution and re-assayed. Dilution factors need to be taken into consideration in calculating the human DJ-1/PARK7 concentration.

Troubleshooting

1. All samples and standards should be assayed in duplicate, using the protocol described in the **Detailed Protocol**. Incubation times or temperatures significantly different from those specified may give erroneous results.
2. Poor duplicates, accompanied by elevated values for wells containing no sample, indicate insufficient washing. If all instructions in the **Detailed Protocol** were followed accurately, such results indicate a need for washer maintenance.
3. Overall low signal may indicate that desiccation of the plate has occurred between the final wash and addition of Substrate Reagent. Do not allow the plate to dry out. Add Substrate Reagent immediately after wash.

Reagent Stability

All of the reagents included in the MBL Research Product **CircuLex Human DJ-1/PARK7 ELISA Kit Ver.2** have been tested for stability. Reagents should not be used beyond the stated expiration date. Upon receipt, kit reagents should be stored at 4°C, except the reconstituted Human DJ-1/PARK7 Standard must be stored at below -70°C. Coated assay plates should be stored in the original foil bag sealed by the zip lock and containing a desiccant pack.

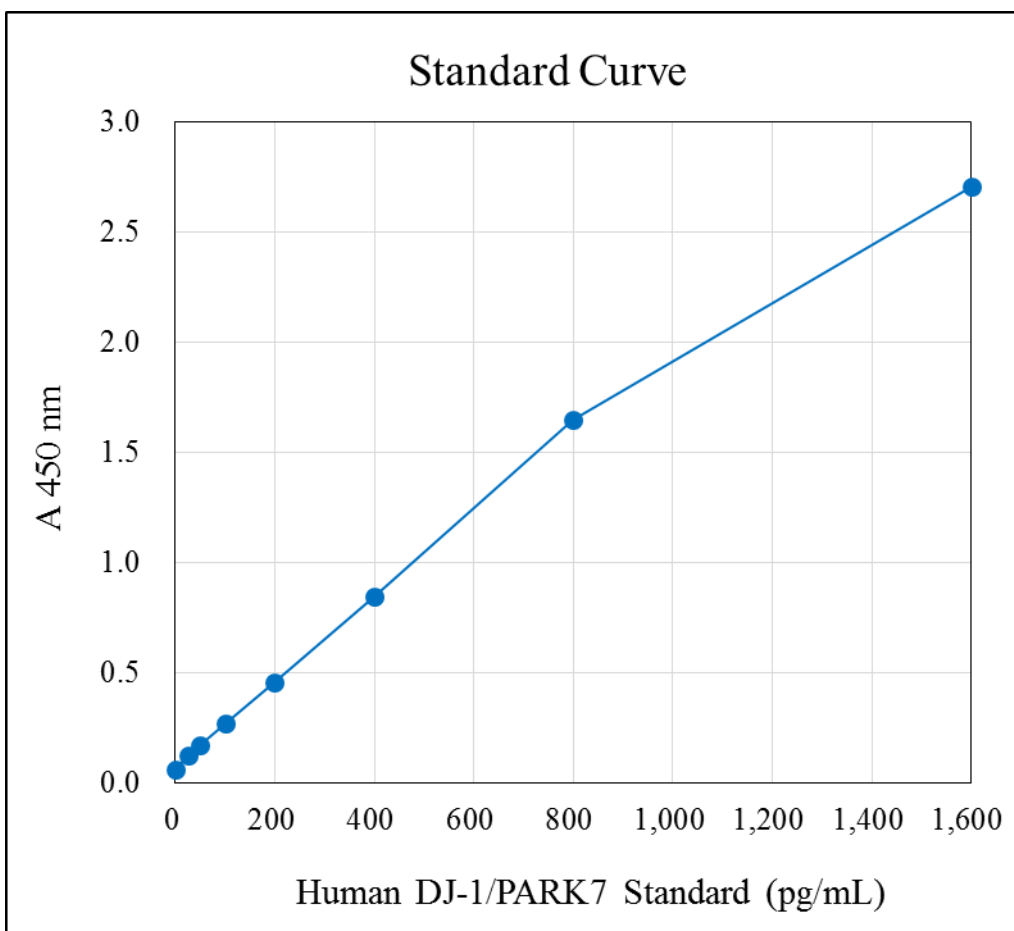
Assay Characteristics

1. Sensitivity

The limit of detection (defined as such a concentration of human DJ-1/PARK7 giving absorbance higher than mean absorbance of blank* plus three standard deviations of the absorbance of blank: A blank + 3SD blank) is better than 8.4 pg/mL of sample.

* Dilution Buffer was pipetted into blank wells.

Typical standard curve



2. Precision

Intra-assay Precision (Precision within an assay)

Four samples* of known concentration were tested six times on one plate to assess intra-assay precision.

- Intra-assay (Within-Run, n=6), CV=2.0 – 5.5%

* Sample: cell lysate

Human DJ-1/PARK7 concentration (ng/mL)

| | Sample 1 | Sample 2 | Sample 3 | Sample 4 |
|---------|----------|----------|----------|----------|
| 1 | 24.4 | 66.0 | 127.2 | 300.8 |
| 2 | 25.0 | 65.3 | 127.0 | 298.8 |
| 3 | 24.0 | 65.4 | 128.7 | 300.9 |
| 4 | 25.4 | 57.5 | 135.5 | 300.0 |
| 5 | 24.6 | 61.9 | 123.1 | 284.9 |
| 6 | 24.5 | 66.8 | 124.4 | 285.9 |
| Max. | 25.4 | 66.8 | 135.5 | 300.9 |
| Min. | 24.0 | 57.5 | 123.1 | 284.9 |
| Average | 24.6 | 63.8 | 127.6 | 295.2 |
| STDEV | 0.5 | 3.5 | 4.4 | 7.6 |
| C.V.(%) | 2.0 | 5.5 | 3.4 | 2.6 |

Inter-assay Precision (Precision between assays)

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

- Inter-assay (Run-to-Run, n=3), CV=4.6 – 8.7%

* Sample: cell lysate

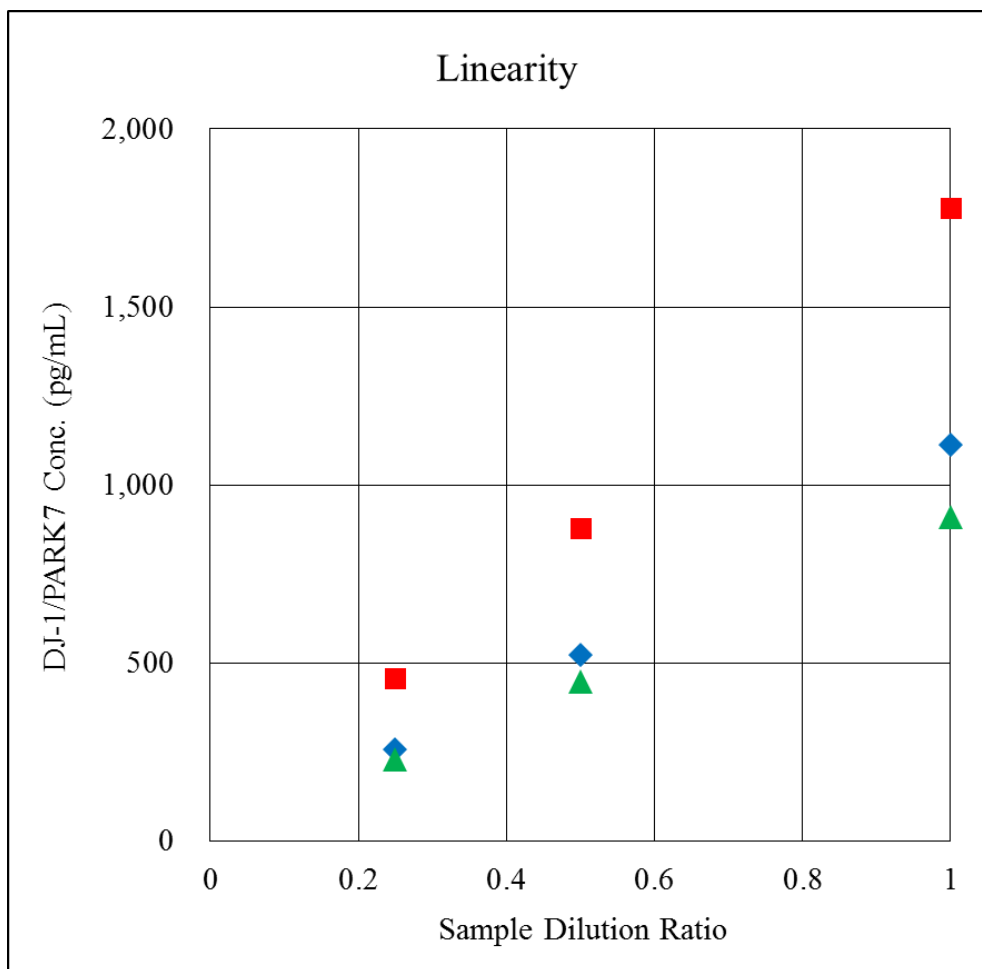
Human DJ-1/PARK7 concentration (ng/mL)

| | Sample 1 | Sample 2 | Sample 3 | Sample 4 |
|---------|----------|----------|----------|----------|
| 1 | 24.7 | 66.2 | 129.6 | 300.9 |
| 2 | 24.5 | 60.5 | 127.0 | 288.3 |
| 3 | 22.7 | 59.7 | 113.7 | 253.7 |
| Max. | 24.7 | 66.2 | 129.6 | 300.9 |
| Min. | 22.7 | 59.7 | 113.7 | 253.7 |
| Average | 24.0 | 62.1 | 123.4 | 281.0 |
| STDEV | 1.1 | 3.6 | 8.5 | 24.4 |
| C.V.(%) | 4.6 | 5.8 | 6.9 | 8.7 |

3. Linearity

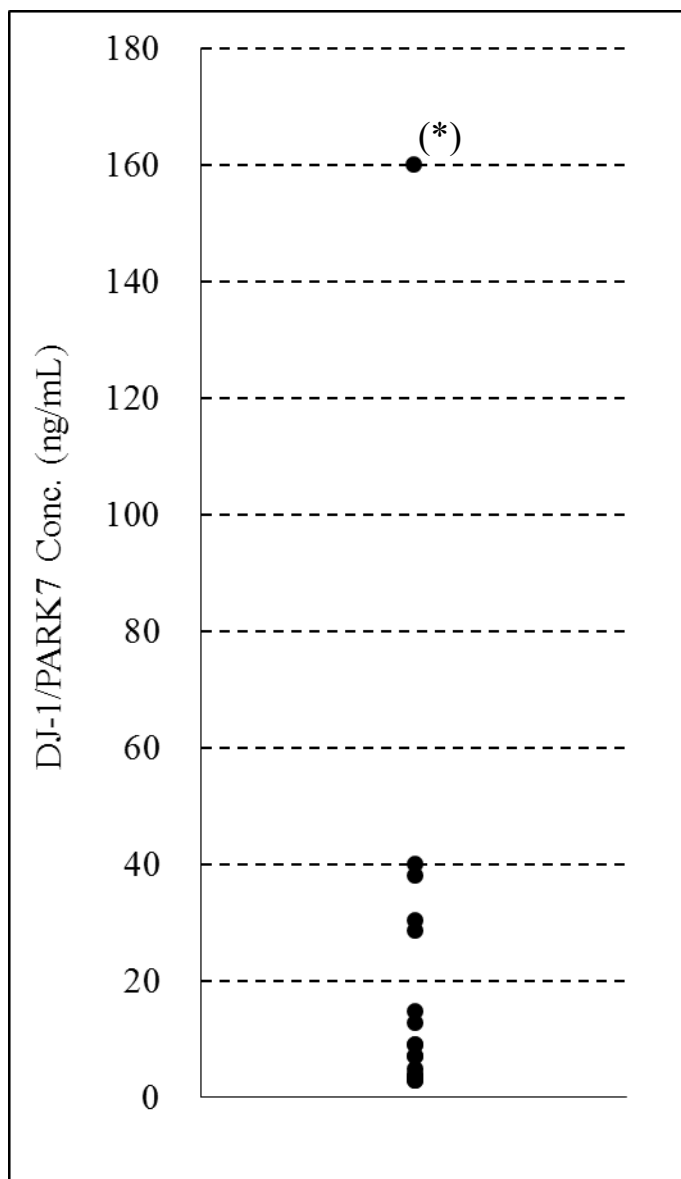
To assess the linearity of the assay, three samples* were serially diluted with the Dilution Buffer to produce samples with values within the dynamic range of the assay.

* Sample: cell lysate



Example of Test Results

Fig.1 Concentrations of DJ-1/PARK7 in healthy Japanese volunteers sera (n = 18)



(*) Prominent hemolysis was observed in the highest serum. Hemolysis may affect the measurement of DJ-1/PARK7 concentrations in serum.

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