

HUMAN SOLUBLE DIPEPTIDYL PEPTIDASE IV (DPPIV)/CD26 ELISA KIT

FOR THE QUANTITATIVE DETERMINATION
OF HUMAN SOLUBLE DPPIV/CD26
CONCENTRATIONS IN CELL CULTURE
SUPERNATES, SERUM AND PLASMA.



THIS IS PROVIDED FOR DEMONSTRATION
ONLY. FOR RESEARCH USE ONLY. NOT FOR
USE IN DIAGNOSTIC PROCEDURES.

PURCHASE INFORMATION:

ELISA NAME	HUMAN SOLUBLE DPPIV/CD26 ELISA
Catalog No.	SK00900-01
Lot No.	
Formulation	96 T
Standard range	31.2-2000 pg/ml
Sensitivity	15.6 pg/ml
Sample Volume	100 µl
Sample Type	Cell Culture Supernates, Serum, Plasma
Dilution factor	300 (Optimal dilutions should be determined by each laboratory for each application)
Specificity	Human soluble DPPIV
Intra-assay Precision	4-6%
Inter-assay Precision	8-10%
Storage	2-8 °C

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INTRODUCTION

Human DPPIV immunoassay is a 3.5 - 4.5 hour solid phase ELISA designed to measure Human DPPIV in Cell Culture Supernates, Serum and EDTA plasma. It contains recombinant Human DPPIV and antibodies raised against this protein. It has been shown to accurately quantify recombinant Human DPPIV. Results obtained with naturally occurring DPPIV samples showed linear curves that were parallel to the standard curves obtained using the kit standards. These results indicate that the immunoassay kit can be used to determine relative mass values for natural Human DPPIV.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. An antibody specific for DPPIV has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any DPPIV present is bound by the immobilized antibody. After washing away any unbound substances, an antibody specific for DPPIV is added to the wells. Following a wash to remove any unbound antibody reagent, Streptavidin HRP conjugate is added to the wells. After washing away any unbound enzyme, a substrate solution is added to the wells and color develops in proportion to the amount of DPPIV bound in the initial step. The color development is stopped and the intensity of the color is measured.

LIMITATIONS OF THE PROCEDURE

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- _ The kit should not be used beyond the expiration date on the kit label.
- _ Do not mix or substitute reagents with those from other lots or sources.
- _ It is important that the Dilution Buffer selected for the standard curve be consistent with the samples being assayed.
- _ If samples generate values higher than the highest standard, dilute the samples with the appropriate Dilution Buffer and repeat the assay.
- _ Any variation in standard diluent, operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.
- _ This assay is designed to eliminate interference by soluble receptors, binding proteins, and other factors present in biological samples. Until all factors

have been tested in the immunoassay, the possibility of interference cannot be excluded.

MATERIALS PROVIDED

DESCRIPTION	CODE	QUANTITY
DPPIV Microplate - 96 well polystyrene microplate (12 strips of 8 wells) coated with an antibody against DPPIV.	900-01-01	1 plate
DPPIV Standard refer to lot specific of recombinant Human DPPIV in a buffered protein base with preservatives; lyophilized.	900-01-02	1 vial
Detection Antibody Concentrate — refer to lot specific of an antibody against DPPIV with preservatives; lyophilized.	900-01-03	1 vial
Positive Control - one vial of recombinant Human DPPIV in a buffered protein base with preservatives; lyophilized.	900-01-04	1 vial
Streptavidin HRP Conjugate -60 µl/vial, 200-fold concentrated solution of Streptavidin HRP conjugate	SAHRP	1 vial
Dilution Buffer - 60 mL of buffered protein based solution with preservatives	DB01	1 bottle
Wash Buffer -50 mL of 10-fold concentrated buffered surfactant, with preservative.	WB01	1 bottle
TMB Substrate Solution - 11 mL of TMB substrate solution	TMB01	1 bottle
Stop Solution - 11 mL of 0.5M HCl	S-STOP	1 bottle
Plate Sealer	EAPS	1 piece
Plastic Pouch	P01	1 piece

STORAGE

Unopened Kit: Unopened Kit: Store at 2 - 8° C for up to 12 months. For longer storage, unopened Standard, Positive Control and Detection Antibody Concentrated should be stored at -20 or -70 °C. Do not use kit past expiration date.

Opened / Reconstituted Reagents: Reconstituted

Standard and Detection Antibody Concentrate Solution SHOULD BE STORED at -20 °C or -70°C for up to one month. Streptavidin - HRP Conjugate 200-fold concentrated and other components may be stored at 2 - 8°C for up to 12 months.

Microplate Wells: Return unused wells to the plastic pouch with the desiccant pack, reseal along entire edge of zip-seal. Microplate may be stored for up to 6 months at 2 - 8° C after opening.

OTHER SUPPLIES REQUIRED

- Microplate reader capable of measuring absorbance at 450 nm, with the correction wavelength set at 540 nm or 570 nm.
- Microplate shaker (250-300rpm).
- Pipettes and pipette tips.
- Deionized or distilled water.
- Squirt bottle, manifold dispenser, or automated microplate washer.
- 100 mL and 500 mL graduated cylinders.

SAMPLE COLLECTION AND STORAGE

Cell Culture Supernates - Remove particulates by centrifugation and assay immediately or aliquot and store samples at $\leq -20^{\circ}$ C. Avoid repeated freeze-thaw cycles.

Plasma - Collect plasma using EDTA as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Aliquot and store samples at -20 °C ~ -70 °C. Avoid repeated freeze-thaw cycles.

Serum - Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at $\leq -20^{\circ}$ C. Avoid repeated freeze-thaw cycles.

Note: Use Aprotinin (enzyme inhibitor) (Code No.: 00700-01-25) for ALL sample collection to prevent sample degradation. 0.5 TIU per ml of sample solution.

SAMPLE PREPARATION

Serum or plasma samples may require 300-fold dilution. The suggested 300-fold dilution is 10 μ l of samples + 90 μ l of Dilution Buffer, following 10 μ l of 10-fold diluted samples + 290 μ l of dilution buffer.

Optimal dilutions should be determined by each laboratory for each application. Use polypropylene test tubes.

REAGENT PREPARATION

Bring all reagents to room temperature before use.

Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Dilute 50 mL of Wash Buffer Concentrate into deionized or distilled water (450 mL) to prepare 500 mL of Wash Buffer.

DPPIV Standard - Refer to vial label for reconstitution volume. Reconstitute the **DPPIV** standard with refer to lot specific of **Dilution Buffer**. Pipette 250 μ l of the appropriate Dilution Buffer into tubes #1 to #6. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The 2000 pg/mL standard serves as the high standard. The appropriate Dilution Buffer serves as the zero standard (0 pg/mL).

TUBE	STANDARD	DILUTION BUFFER	CONCENTRATION
Stock	powder	Refer to lot specific	2000 pg/ml
# 1	250 μ l of stock	250 μ l	1000 pg/ml
# 2	250 μ l of 1	250 μ l	500 pg/ml
# 3	250 μ l of 2	250 μ l	250 pg/ml
# 4	250 μ l of 3	250 μ l	125 pg/ml
# 5	250 μ l of 4	250 μ l	62.5 pg/ml
# 6	250 μ l of 5	250 μ l	31.25 pg/ml

Detection Antibody - Reconstitute the **Detection Antibody Concentrate** with refer to lot specific of Dilution Buffer to produce a concentrated stock solution. Pipette refer to lot specific of the appropriate Dilution Buffer into the 15 ml centrifuge tube and transfer refer to lot specific of concentrated stock solution to prepare working solution.

Streptavidin HRP Conjugate - Pipette 11.94 mL of Dilution Buffer into a 15 ml centrifuge tube and transfer 60 μ l of 200-fold concentrated stock solution to prepare working solution. *Note: 1 x working solution of Streptavidin-HRP Conjugate should be used within a few days.*

Positive Control- Reconstitute the positive control with refer to lot specific of Dilution Buffer to make positive control solution.

ASSAY PROCEDURE

Bring all reagents and samples to room temperature before use. It is recommended that blank, standards, positive control and samples be assayed in duplicate.

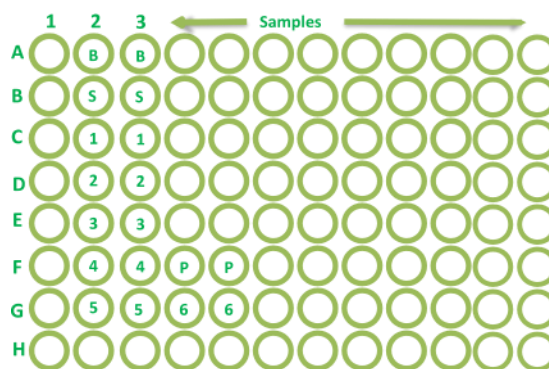
1. Prepare all reagents and working standards as directed in the previous sections.
2. Remove excess micro-plate strips from the plate frame, return them to the plastic pouch with the desiccant pack, reseal.
3. Add 100 µL of **Dilution Buffer** to Blank wells (A2, A3).
4. Add 100 µL of Standard (from B2, B3 to G2, G3, and G4, G5), samples, or positive control (F4, F5) per well. Cover with the plate sealer. Incubate for 2 hours at room temperature on a plate shaker. A plate layout is provided to record standards and samples assayed.
5. Aspirate each well and wash, repeating the process three times for a total of four washes. Wash by filling each well with Wash Buffer (300 µL) using a squirt bottle, manifold dispenser, 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.
6. Add 100 µL of Detection Antibody working solution to each well. Cover with sealer. Incubate for 2 hours on micro-plate shaker at room temperature.
7. Repeat the aspiration/wash as in step 5.
8. Add 100 µL of **Streptavidin HRP Conjugate working solution** to each well. Incubate for 60 minutes on micro-plate shaker at room temperature.
9. Repeat the aspiration/wash as in step 5.
10. Add 100 µL of Substrate Solution to each well. Incubate for refer to lot specific minutes at room temperature. **Protect from light.**
11. Add 100 µL of Stop Solution to each well. The color in the wells should change from blue to yellow. If the color in the wells is green, or if the color change does not appear uniform, gently tap the plate to ensure thorough mixing.
12. Determine the optical density of each well within 15 minutes, using a micro-plate reader set to 450 nm.

CALCULATION OF RESULTS

Average the duplicate readings for each standard, positive control, and sample and subtract the

average zero standard optical density. Create a standard curve by reducing the data using computer software capable of generating a log-log curve fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the DPPIV concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data.

If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.



TYPICAL DATA

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

DPPIV (PG/ML)	CORRECTED (450NM)
Blank	0 (0.06)
31.25	0.030
62.5	0.055
125	0.108
250	0.209
500	0.414
1000	0.722
2000	1.303

CALIBRATION

This immunoassay is calibrated against a recombinant Human DPPIV.

SENSITIVITY

Twenty-five assays were evaluated and the minimum detectable dose (MDD) of DPPIV was 15.6 pg/mL.

SPECIFICITY

PROTEINS	CROSSREACTIVITY (%)
Human DPPIV	100
Human ECE1	0
Human ACE	0
Human ECE2	0
Human ACE-2	0
Mouse DPPIV	0

LINEARITY

To assess the linearity of the assay, pooled research human **EDTA plasma** samples were diluted with Dilution Buffer and assayed.

DILUTION FACTOR	ASSAYED (PG/ML)	FINAL (PG/ML)	RECOVERY (%)
300 X	1910.242	573072.6	100
600 X	1106.605	663963	115

To assess the linearity of the assay, pooled research human serum samples were diluted with Dilution Buffer and assayed.

DILUTION FACTOR	ASSAYED (PG/ML)	FINAL (PG/ML)	RECOVERY (%)
300 X	2144.499	643349.7	100
600 X	1162.298	697618.8	108

SUMMARY OF ASSAY PROCEDURE

PREPARE REAGENTS, SAMPLES AND STANDARDS
↓
Add 100 µl of standard, samples, positive control to each well. Incubate for 2 hours on the plate shaker at RT.
↓
Aspirate and wash 4 times.
↓
Add 100 µl Detection Antibody working solution to each well. Incubate 2 hour on the plate shaker at RT.
↓
Aspirate and wash 4 times.
↓
Add 100 µl Streptavidin HRP conjugate working

solution to each well. Incubate 60 min on the plate shaker at RT. Protect from light.
↓
Aspirate and wash 4 times.
↓
Add 100 µl Substrate Solution to each well. Incubate refer to lot specific on the plate shaker. Protect from light.
↓
Add 100 µl Stop Solution to each well. Read 450nm within 15 min