

Quantikine[®]

Human DPPIV/CD26 Immunoassay

Catalog Number DC260

For the quantitative determination of human Dipeptidyl Peptidase IV (DPPIV) concentrations in cell culture supernates, serum, plasma, saliva, and urine.

This package insert must be read in its entirety before using this product.

**FOR RESEARCH USE ONLY.
NOT FOR USE IN DIAGNOSTIC PROCEDURES.**

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INTRODUCTION

Dipeptidyl Peptidase IV (DPPIV), also known as T-cell activation antigen CD26 and adenosine deaminase (ADA) complexing protein 2, is a serine protease that releases Xaa-Pro dipeptides from the N-terminus of oligo- and polypeptides (1). It is a type II membrane protein consisting of a small cytoplasmic tail, a transmembrane region, and a large extracellular domain. The extracellular domain contains glycosylation sites, a cysteine-rich region, and the catalytic active site (Ser, Asp and His charge relay system). In the native state, DPPIV/CD26 is present as a non-covalently linked homodimer on the surface of a variety of cell types. The soluble form is also present in human serum and other body fluids. The form purified from human serum or seminal fluid corresponds to the intact extracellular domain (2).

DPPIV/CD26 plays an important role in many physiological and pathological processes. It interacts with ADA and CD45, providing a co-stimulating signal to the CD3/T-cell receptor complex (3). It cleaves many chemokines with Xaa-Pro at their N-terminus, altering their receptor specificity and biological function (3). It degrades many peptide hormones, such as glucagon-like peptide-1, shorting their bioactivity. DPPIV inhibitors are being developed to extend their bioactivity and currently being tested in late-stage clinical trials for the treatment of type 2 diabetes (4). DPPIV truncates the N-terminus of procalcitonin, a marker for systemic bacterial and fungal infections (5). DPPIV/CD26 interacts with HIV-1 Tat protein and its binding to ADA is inhibited by HIV envelop protein gp120 (6).

The Quantikine DPPIV Immunoassay is a 4.5 hour solid-phase ELISA designed to measure human DPPIV/CD26 in cell culture supernates, serum, plasma, saliva, and urine. It contains NS0-expressed recombinant human DPPIV, and antibodies raised against the recombinant factor. Results obtained using natural human DPPIV showed dose-response curves that were parallel to the standard curves obtained using the recombinant Quantikine kit standards. These results indicate that the Quantikine DPPIV kit can be used to determine relative mass values for naturally occurring DPPIV.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal 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 enzyme-linked polyclonal antibody specific for DPPIV is added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, 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

- FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
- 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.
- If samples generate values higher than the highest standard, further dilute the samples with the appropriate Calibrator Diluent 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 Quantikine Immunoassay, the possibility of interference cannot be excluded.

REAGENTS

DPPIV Microplate (Part 892951) - 96 well polystyrene microplate (12 strips of 8 wells) coated with a rat monoclonal antibody against DPPIV.

DPPIV Conjugate (Part 892952) - 21 mL of polyclonal antibody against DPPIV conjugated to horseradish peroxidase with preservatives.

DPPIV Standard (Part 892953) - 200 ng of recombinant human DPPIV in a buffer with preservatives, lyophilized.

Assay Diluent RD1-57 (Part 895207) - 11 mL of a buffer with preservatives.

Calibrator Diluent RD5-33 (Part 895813) - 3 vials (21 mL/vial) of a buffered protein base with preservatives. *For serum/plasma samples.*

Calibrator Diluent RD5K (Part 895119) - 21 mL of a animal serum with preservatives. *For cell culture supernate, saliva, and urine samples.*

Wash Buffer Concentrate (Part 895003) - 21 mL of a 25-fold concentrated solution of buffered surfactant with preservatives.

Color Reagent A (Part 895000) - 12.5 mL of stabilized hydrogen peroxide.

Color Reagent B (Part 895001) - 12.5 mL of stabilized chromogen (tetramethylbenzidine).

Stop Solution (Part 895032) - 6 mL of 2 N sulfuric acid.

Plate Covers - 4 adhesive strips.

STORAGE

Unopened Kit	Store at 2 - 8° C. Do not use past kit expiration date.	
Opened/ Reconstituted Reagents	Diluted Wash Buffer	May be stored for up to 1 month at 2 - 8° C.*
	Stop Solution	
	Assay Diluent RD1-57	
	Calibrator Diluent RD5-33	
	Calibrator Diluent RD5K	
	Conjugate	
	Unmixed Color Reagent A	
	Unmixed Color Reagent B	
	Standard	
	Microplate Wells	Return unused wells to the foil pouch containing the desiccant pack, reseal along entire edge of zip-seal. May be stored for up to 1 month at 2 - 8° C.*

*Provided this is within the expiration date of the kit.

OTHER SUPPLIES REQUIRED

- Microplate reader capable of measuring absorbance at 450 nm, with the correction wavelength set at 540 nm or 570 nm.
- Pipettes and pipette tips.
- Deionized or distilled water.
- Squirt bottle, manifold dispenser, or automated microplate washer.
- 500 mL graduated cylinder.
- Collection device for saliva samples that has no protein binding or filtering capabilities such as AccuSorb™ or equivalent.
- Human DPPIV Controls (optional; available from R&D Systems).

PRECAUTIONS

The Stop Solution provided with this kit is an acid solution. Wear eye, hand, face, and clothing protection when using this material.

DPPIV is detectable in saliva. Take precautionary measures to prevent contamination of kit reagents while running this assay.

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.

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

Plasma - Collect plasma using heparin or EDTA as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at $\leq -20^{\circ}$ C. Avoid repeated freeze-thaw cycles.

Note: *Citrate plasma has not been validated for use in this assay.*

Saliva - Collect saliva using a collection device such as an AccuSorb™ or equivalent. Assay immediately or aliquot and store samples at $\leq -20^{\circ}$ C. Avoid repeated freeze-thaw cycles.

Note: *Saliva collector must not have any protein binding or filtering capabilities.*

Urine - Aseptically collect the first urine of the day (mid-stream), voided directly into a sterile container. Centrifuge to remove particulate matter, assay immediately or aliquot and store at $\leq -20^{\circ}$ C. Avoid repeated freeze-thaw cycles. For more information on collecting and handling urine specimens, refer to the National Committee for Clinical Laboratory Standards (NCCLS) publication GP16-T.

SAMPLE PREPARATION

Serum/plasma samples require a 100-fold dilution. A suggested 100-fold dilution is 10 μ L sample + 990 μ L Calbrator Diluent RD5-33.

Saliva samples require a 4-fold dilution. A suggested 4-fold dilution is 100 μ L sample + 300 μ L Calbrator Diluent RD5K.

REAGENT PREPARATION

Bring all reagents to room temperature before use.

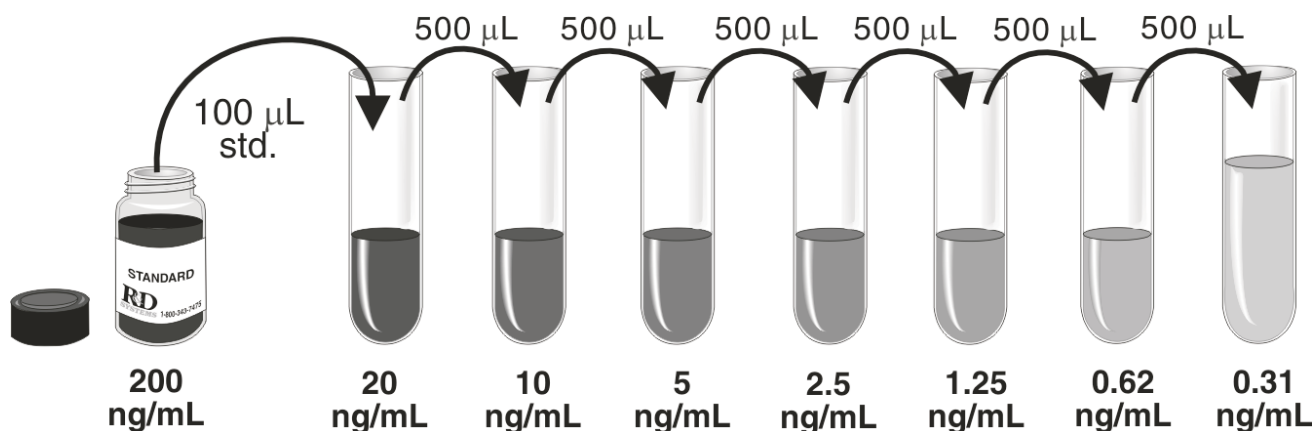
Note: High concentrations of DPPIV are found in saliva. We recommend using a face mask and gloves to protect kit reagents from contamination.

Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Dilute 20 mL of Wash Buffer Concentrate into deionized or distilled water to prepare 500 mL of Wash Buffer.

Substrate Solution - Color Reagents A and B should be mixed together in equal volumes within 15 minutes of use. Protect from light. 200 μ L of the resultant mixture is required per well.

DPPIV Standard - Reconstitute the DPPIV Standard with 1.0 mL of deionized or distilled water. This reconstitution produces a stock solution of 200 ng/mL. Mix the standard to ensure complete reconstitution and allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions.

Pipette 900 μ L of Calibrator Diluent RD5K (for cell culture supernate, saliva and urine samples) or Calibrator Diluent RD5-33 (for serum/plasma samples) into the 20 ng/mL tube. Pipette 500 μ L into the remaining tubes. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The 20 ng/mL standard serves as the high standard. The appropriate Calibrator Diluent serves as the zero standard (0 pg/mL).



ASSAY PROCEDURE

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

Note: *High concentrations of DPPIV are found in saliva. We recommend using a face mask and gloves to protect kit reagents from contamination.*

1. Prepare all reagents, working standards and samples as directed in the previous sections.
2. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, reseal.
3. Add 100 μL of Assay Diluent RD1-57 to each well.
4. Add 50 μL of Standard, control, or sample* per well. Cover with the adhesive strip provided. Incubate for 2 hours at room temperature. 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 (400 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 200 μL of DPPIV Conjugate to each well. Cover with a new adhesive strip. Incubate for 2 hours at room temperature on the benchtop.
7. Repeat the aspiration/wash as in step 5.
8. Add 200 μL of Substrate Solution to each well. Incubate for 30 minutes at room temperature. **Protect from light.**
9. Add 50 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 the color change does not appear uniform, gently tap the plate to ensure thorough mixing.
10. Determine the optical density of each well within 30 minutes, using a microplate reader set to 450 nm. If wavelength correction is available, set to 540 nm or 570 nm. If wavelength correction is not available, subtract readings at 540 nm or 570 nm from the readings at 450 nm. This subtraction will correct for optical imperfections in the plate. Readings made directly at 450 nm without correction may be higher and less accurate.

*Serum/plasma/saliva samples require dilution. See Sample Preparation section.

ASSAY PROCEDURE SUMMARY

1. Prepare reagents, samples, and standards as instructed.



2. Add 100 μL Assay Diluent RD1-57 to each well.



3. Add 50 μL Standard, control, or sample* to each well. Incubate 2 hrs. at RT.



4. Aspirate and wash 4 times.



5. Add 200 μL Conjugate to each well. Incubate 2 hrs. at RT.



6. Aspirate and wash 4 times.



7. Add 200 μL Substrate Solution to each well. Incubate 30 min. **Protect from light.**



8. Add 50 μL Stop Solution to each well. Read at 450 nm within 30 min.
 λ correction 540 or 570 nm

*Serum/plasma/saliva samples require dilution.
See Sample Preparation section.

CALCULATION OF RESULTS

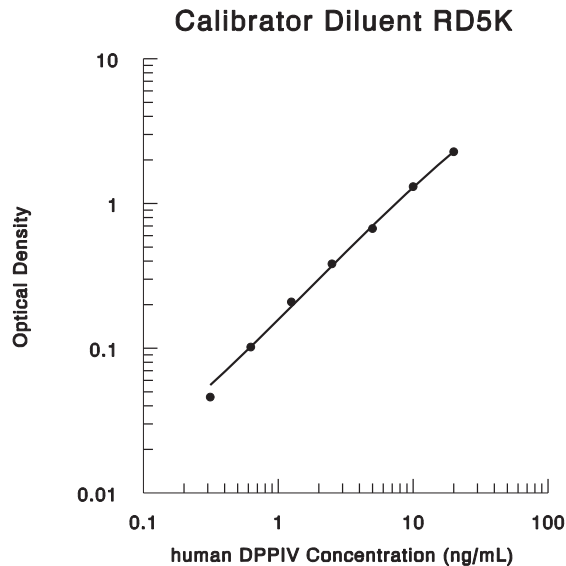
Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density.

Create a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) 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.

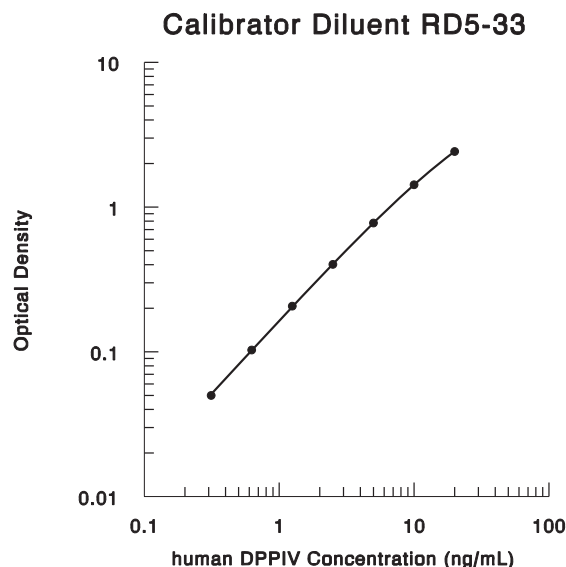
If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

TYPICAL DATA

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



ng/mL	O.D.	Average	Corrected
0	0.010 0.010 0.055	0.010	—
0.31	0.056 0.110	0.056	0.046
0.62	0.114 0.214	0.112	0.102
1.25	0.223 0.385	0.219	0.209
2.5	0.401 0.656	0.393	0.383
5	0.707 1.307	0.682	0.672
10	1.330 2.284	1.319	1.309
20	2.297	2.291	2.281



ng/mL	O.D.	Average	Corrected
0	0.010 0.010 0.057	0.010	—
0.31	0.062 0.111	0.060	0.050
0.62	0.115 0.211	0.113	0.103
1.25	0.222 0.407	0.217	0.207
2.5	0.416 0.762	0.412	0.402
5	0.812 1.428	0.787	0.777
10	1.445 2.432	1.437	1.427
20	2.436	2.434	2.424

TECHNICAL HINTS

- When mixing or reconstituting protein solutions, always avoid foaming.
- To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.
- When using an automated plate washer, adding a 30 second soak period following the addition of wash buffer, and/or rotating the plate 180 degrees between wash steps may improve assay precision.
- To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
- Substrate Solution should remain colorless until added to the plate. Keep Substrate Solution protected from light. Substrate Solution should change from colorless to gradations of blue.
- Stop Solution should be added to the plate in the same order as the Substrate Solution. The color developed in the wells will turn from blue to yellow upon addition of the Stop Solution. Wells that are green in color indicate that the Stop Solution has not mixed thoroughly with the Substrate Solution.

PRECISION

Intra-assay Precision (Precision within an assay)

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

Inter-assay Precision (Precision between assays)

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

Serum/Plasma Assay

Sample	Intra-assay Precision			Inter-assay Precision		
	1	2	3	1	2	3
n	20	20	20	40	40	40
Mean (pg/mL)	2.32	8.78	15.8	2.43	8.53	15.9
Standard deviation	0.142	0.354	1.15	0.207	0.777	1.30
CV (%)	6.1	4.0	7.3	8.5	9.1	8.2

Cell Culture Supernate/Saliva/Urine Assay

Sample	Intra-assay Precision			Inter-assay Precision		
	1	2	3	1	2	3
n	20	20	20	40	40	40
Mean (pg/mL)	2.16	8.15	14.1	2.22	8.25	14.6
Standard deviation	0.105	0.280	0.916	0.140	0.695	1.18
CV (%)	4.9	3.4	6.5	6.3	8.4	8.1

RECOVERY

The recovery of DPPIV spiked to levels throughout the range of the assay in various matrices was evaluated.

Sample	Average % Recovery	Range
Cell culture media (n=4)	101	87 - 113%
Urine (n=4)	101	88 - 113%

LINEARITY

To assess the linearity of the assay, samples containing and/or spiked with high concentrations of DPPIV were serially diluted with the appropriate Calibrator Diluent to produce samples with values within the dynamic range of the assay.

		Cell culture supernates (n=4)	Serum* (n=4)	Heparin plasma* (n=4)	EDTA plasma* (n=4)	Urine (n=3)	Saliva (n=4)
1:2	Average % of Expected	98	104	105	105	101	101
	Range (%)	87 - 110	101 - 107	102 - 108	99 - 108	99 - 102	99 - 102
1:4	Average % of Expected	96	107	105	105	101	103
	Range (%)	84 - 115	104 - 112	100 - 108	101 - 107	101 - 102	100 - 105
1:8	Average % of Expected	93	105	103	105	100	101
	Range (%)	83 - 112	100 - 110	98 - 108	101 - 110	97 - 103	94 - 107
1:16	Average % of Expected	95	103	98	100	—	95
	Range (%)	86 - 102	97 - 108	97 - 99	95 - 105		91 - 99

*Samples were diluted prior to assay as instructed in the Sample Preparation section.

SENSITIVITY

Ninety-nine assays were evaluated and the minimum detectable dose (MDD) of DPPIV ranged from 0.006 - 0.072 ng/mL. The mean MDD was 0.016 ng/mL.

The MDD was determined by adding two standard deviations to the mean optical density value of twenty zero standard replicates and calculating the corresponding concentration.

CALIBRATION

This immunoassay is calibrated against a highly purified NS0-expressed recombinant human DPPIV produced at R&D Systems.

SAMPLE VALUES

Serum/Plasma/Urine/Saliva - Samples were evaluated for the presence of DPPIV in this assay.

Sample Type	Mean (ng/mL)	Range (ng/mL)	Standard Deviation
Serum (n=36)	415	197 - 615	96.0
EDTA plasma (n=36)	391	187 - 604	85.4
Heparin plasma (n=36)	406	159 - 588	91.8
Urine (n=10)	6.06	2.26 - 13.3	3.35
Saliva (n=10)	32.6	13.0 - 69.9	17.9

Cell Culture Supernates -

Human peripheral blood cells (1×10^6 cells/mL) were cultured in DMEM supplemented with 5% fetal calf serum, 50 μ M β -mercaptoethanol, 2 mM L-glutamine, 100 U/mL penicillin, and 100 μ g/mL streptomycin sulfate. Cells were cultured unstimulated or stimulated with 10 μ g/mL PHA. Aliquots of the culture supernate were removed and assayed for levels of natural human DPPIV.

Condition	Day 1 (ng/mL)	Day 6 (ng/mL)
Unstimulated	ND	ND
Stimulated	ND	0.697

ND = Non-detectable

HepG2 - Human liver cell line cells were cultured in MEM supplemented with 5% fetal calf serum until confluent and stimulated with 50 ng/mL PMA for 24 hours. An aliquot of the cell culture supernate was removed, assayed for levels of natural human DPPIV, and measured 10.6 ng/mL.

SPECIFICITY

This assay recognizes recombinant and natural human DPPIV. The factors listed below were prepared at 200 ng/mL in Calibrator Diluents RD5K and RD5-33 and assayed for cross-reactivity. Preparations of the following factors at 20 ng/mL in a mid-range recombinant human DPPIV control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant human:

ACE
ACE-2
DPP-6
ECE-1
ECE-2
Neprilysin

Recombinant mouse:

CD26

REFERENCES

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2. Durinx, C. *et al.* (2000) *Eur. J. Biochem.* **267**:5608.
3. Sato, K. and N.H. Dang (2003) *Int. J. Oncol.* **22**:481.
4. Nielsen, L.L. (2005) *DDT* **10**:703.
5. Wrenger, S. *et al.* (2000) *FEBS Lett.* **466**:155.
6. Weihofen, W.A. *et al.* (2004) *J. Biol. Chem.* **279**:43330.

AccuSorb is a trademark of Avitar, Inc.

PLATE LAYOUT

Use this plate layout as a record of standards and samples assayed.

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

NOTES