Version 19-04/24

**User's Manual**

# Human DPPIV/CD26 ELISA Kit

REF **DEIA2831**

Σ **96T**

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This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

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# PRODUCT INFORMATION

## Intended Use

The Human DPPIV/CD26 ELISA Kit kit is an in vitro enzyme-linked immunosorbent assay for the quantitative measurement of human CD26 in serum, plasma, cell culture supernatants and urine.

## General Description

Dipeptidyl Peptidase IV (DPPIV; also known as 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. 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 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.

DPPIV 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. It cleaves many chemokines with Xaa-Pro at their N-terminus, altering their receptor specificity and biological function. 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. DPPIV truncates the N-terminus of procalcitonin, a marker for systemic bacterial and fungal infections. DPPIV interacts with HIV-1 Tat protein and its binding to ADA is inhibited by HIV envelop protein gp120.

## Principles of Testing

This assay employs an antibody specific for human CD26 coated on a 96-well plate. Standards and samples are pipetted into the wells and CD26 present in a sample is bound to the wells by the immobilized antibody. The wells are washed and biotinylated anti-human CD26 antibody is added. After washing away unbound biotinylated antibody, HRP-conjugated streptavidin is pipetted to the wells. The wells are again washed, a TMB substrate solution is added to the wells and color develops in proportion to the amount of CD26 bound. The Stop Solution changes the color from blue to yellow, and the intensity of the color is measured at 450 nm.

## Reagents And Materials Provided

1. CD26 Microplate (Item A): 96 wells (12 strips x 8 wells) coated with anti-human CD26.
2. Wash Buffer Concentrate (20x) (Item B): 25 ml of 20x concentrated solution.
3. Standards (Item C): 2 vials of recombinant human CD26.
4. Assay Diluent A (Item D): 30 ml diluent buffer, 0.09% sodium azide as preservative. For Standard/Sample (serum/plasma) diluent.
5. Assay Diluent B (Item E): 15 ml of 5x concentrated buffer. For Standard/Sample (cell culture medium/urine)

diluent.

6. Detection Antibody CD26 (Item F): 2 vial of biotinylated anti-human CD26 (each vial is enough to assay half microplate).
7. HRP-Streptavidin Concentrate (Item G): 200 µl 500x concentrated HRP-conjugated streptavidin.
8. TMB One-Step Substrate Reagent (Item H): 12 ml of 3,3',5,5'-tetramethylbenzidine (TMB) in buffer solution.
9. Stop Solution (Item I): 8 ml of 0.2 M sulfuric acid.

## Materials Required But Not Supplied

1. Microplate reader capable of measuring absorbance at 450 nm.
2. Precision pipettes to deliver 2 µl to 1 ml volumes.
3. Adjustable 1-25 ml pipettes for reagent preparation.
4. 100 ml and 1 liter graduated cylinders.
5. Absorbent paper.
6. Distilled or deionized water.
7. Log-log graph paper or computer and software for ELISA data analysis.
8. Tubes to prepare standard or sample dilutions.

## Storage

May be stored for up to 6 months at 2 to 8°C from the date of shipment. Standard (recombinant protein) should be stored at -20°C or -80°C (recommended at -80°C) after reconstitution. Opened Microplate Wells or reagents may be stored for up to 1 month at 2 to 8°C. Return unused wells to the pouch containing desiccant pack, reseal along entire edge.

Note: the kit can be used within one year if the whole kit is stored at -20°C. Avoid repeated freeze-thaw cycles.

## 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 A (Item D) should be used for dilution of serum/plasma samples. 1x Assay Diluent B (Item E) should be used for dilution of culture supernatants and urine.

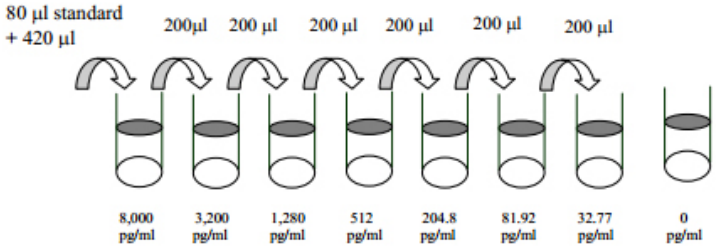
Suggested dilution for normal serum/plasma: 500-5,000 fold*.

* Please note that levels of the target protein may vary between different specimens. Optimal dilution factors for each sample must be determined by the investigator.

For example, add 1 µl of serum/plasma into a tube 999 µl Assay Diluent A to prepare a 1,000-fold diluted sample. Mix well.

3. Assay Diluent B should be diluted 5-fold with deionized or distilled water before use.
4. Preparation of standard: **Briefly spin the vial of Item C** and then add 400 µl Assay Diluent A (for serum/plasma samples) or 1x Assay Diluent B (for cell culture supernates/urine) into Item C vial to prepare

a 50 ng/ml standard. **Dissolve the powder thoroughly by a gentle mix**. Add 80 µl CD26 standard (50 ng/ml) from the vial of Item C, into a tube with 420 µl Assay Diluent A or 1x Assay Diluent B to prepare a 8,000 pg/ml standard solution. Pipette 300 µl Assay Diluent A or 1x Assay Diluent B into each tube. Use the 8,000 pg/ml standard solution to produce a dilution series (shown below). Mix each tube thoroughly before the next transfer. Assay Diluent A or 1x Assay Diluent B serves as the zero standard (0 pg/ml).




+CR2

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 into the vial to prepare a detection antibody concentrate. Pipette up and down to mix gently (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 500-fold with 1x Assay Diluent B.

For example: Briefly spin the vial (Item G) and pipette up and down to mix gently . Add 20 µl of HRP-Streptavidin concentrate into a tube with 10 ml 1x Assay Diluent B to prepare a 500-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 µl 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 the solution and wash 4 times with 1x Wash Solution. Wash by filling each well with Wash Buffer (300 µl) using a multi-channel Pipette 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.
4. Add 100 µl 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 µl 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 µl of TMB One-Step Substrate Reagent (Item H) to each well. Incubate for 30 minutes at room temperature in the dark with gentle shaking.

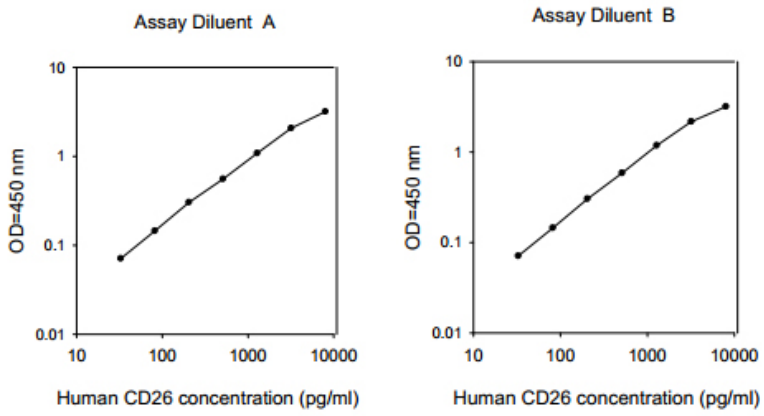
9. Add 50 µl of Stop Solution (Item I) to each well. Read at 450 nm immediately.

## Calculation

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.

## Typical Standard Curve

These standard curves are for demonstration only. A standard curve must be run with each assay.




## Detection Range

25 pg/mL - 8000 pg/mL

## Sensitivity

25 pg/mL

## Specificity

This ELISA kit shows no cross-reactivity with the following cytokines tested: human Angiogenin, BDNF, BLC, CNTF, ENA-78, FGF-4, IL-1α, IL-1β, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-11, IL-12 p70, IL-12 p40, IL-13, IL-15, IL-309, IP-10, FGF-4, FGF-6, FGF-7, G-CSF, GDNF, GM-CSF, IFN-γ, IGFBP-2, IGF-BP-3, IGF-BP-4, Leptin (OB), MCP-1, MCP-2, MCP-3, MDC, MIF, MIG, MIP-1α, MIP-1 β, MIP-1δ, PARC, PDGF, RANTES, SCF,SDF-1alpha, TARC, TGF-β, TIMP-1, TIMP-2, TNF-α, TNF-β, TPO, VEGF.

## Linearity

Sample Type Average % of Expected Range (%)

| | | |
|---|---|---|
| Serum | 97.20 (1:2 dilution); 82.47 (1:4 dilution) | 89-105 (1:2 dilution); 74-90 (1:4 dilution) |
| Plasma | 90.13 (1:2 dilution); 84.78 (1:4 dilution) | 77-103 (1:2 dilution); 79-89 (1:4 dilution) |
| Cell culture media | 94.52 (1:2 dilution); 95.87 (1:4 dilution) | 87-101 (1:2 dilution); 83-108 (1:4 dilution) |

## Recovery

Recovery was determined by spiking various levels human CD26 into human serum, plasma and cell culture media. Mean recoveries are as follows:

| Sample Type | Average %Recovery | Range (%) |
|---|---|---|
| Serum | 122.8 | 118-134 |
| Plasma | 114.4 | 106-122 |
| Cell culture media | 119.5 | 105-130 |

## Reproducibility

Intra-Assay: CV<10%

Inter-Assay: CV<12%

## References

1 Jean A. Nemzek, Javed Siddiqui, Daniel G. Remick: Development and optimization of cytokine ELISAs using commercial antibody pairs. Journal of Immunological Methods, 2001; 255:149–157.

2 Zhenyu Ju, Hong Jiang, Maike Jaworski, Chozhavendan Rathinam, Anne Gompf, Christoph Klein, Andreas Trumpp & K Lenhard Rudolph: Telomere dysfunction induces environmental alterations limiting hematopoietic stem cell function and Engraftment. Nature Medicine, 2007; 13: 742-747.3.

3 Anyuan Sun, Haiming Wei, Rui Sun, Weihua Xiao,Yongguang Yang, and Zhigang Tian: Human Interleukin-15 Improves Engraftment of Human T Cells in NOD-SCID Mice. Clinical and Vaccine Immunology, 2006; 13 (2): 227–234.4.