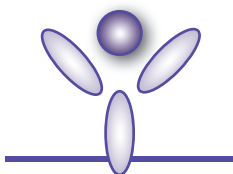




## **LEGEND MAX<sup>™</sup>**

ELISA Kit



## **Human CD26 (DPP4)**

Cat. No. 449307

ELISA Kit for Accurate Quantitation of  
Human CD26 (DPP4)  
in Serum, Plasma, Saliva, Urine,  
and Cell Culture Supernatant

BioLegend, Inc.  
[biolegend.com](http://biolegend.com)

***It is highly recommended that this manual be read in its entirety before using this product. Do not use this kit beyond the expiration date.***

*For Research Purposes Only. Not for use in diagnostic or therapeutic procedures. Purchase does not include or carry the right to resell or transfer this product either as a stand-alone product or as a component of another product. Any use of this product other than the permitted use without the express written authorization of BioLegend is strictly prohibited.*



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# LEGEND MAX™ Human CD26 (DPP4) ELISA Kit

## Introduction:

CD26, also known as Dipeptidyl peptidase-4 (DPP4), is a serine exopeptidase expressed in many different cell types. It can exist as both a membrane protein and an active soluble form. CD26 is involved in many biological processes including but not limited to cell signaling, immune regulation, and apoptosis. For instance, it can act as a co-stimulatory receptor for T-cells activation, while being able to cleave multiple chemokines and peptide hormones such as glucagon, shortening their activity. Due to its abundance and multifunctional characteristic, CD26 can be a biomarker for multiple diseases such as immune dysfunction, obesity, diabetes, and cancer.

The LEGEND MAX™ Human CD26 (DPP4) ELISA Kit is a Sandwich Enzyme-Linked Immunosorbent Assay (ELISA) with a 96-well strip plate that is pre-coated with anti-human CD26 (DPP4) antibody. The detection antibody is a biotinylated anti-human CD26 (DPP4) antibody. This kit is specifically designed for the accurate quantitation of human CD26 (DPP4) in serum, plasma, saliva, urine, and cell culture supernatant. It is analytically validated with ready-to-use reagents.

## Materials Provided:

Description	Quantity	Volume	Part #
Human CD26 (DPP4) pre-coated 96-well Strip Microplate	1 plate		750003049
Human CD26 (DPP4) Detection Antibody	1 bottle	12 mL	750003051
Human CD26 (DPP4) Lyophilized Standard	1 vial	lyophilized	750002874
Avidin HRP	1 bottle	12 mL	77897
Assay Buffer B	1 bottle	25 mL	79128
Wash Buffer (20x)	1 bottle	50 mL	78233
Substrate Solution D	1 bottle	12 mL	78115
Stop Solution	1 bottle	12 mL	79133
Plate Sealers	4 sheets		78101

**Materials to be Provided by the End-User:**

- Microplate reader able to measure absorbance at 450 nm
- Adjustable pipettes to measure volumes ranging from 1 µL to 1,000 µL
- Deionized water
- Wash bottle or automated microplate washer
- Log-Log graph paper or software for data analysis
- Polypropylene tubes to prepare standard dilutions
- Timer
- Plate Shaker
- Polypropylene vials

**Storage Information:**

Store unopened kit components between 2°C and 8°C. Do not use this kit beyond its expiration date.

Opened or Reconstituted Components	
Microplate wells	If not all microplate strips are used, remove the excess strips by pressing up from underneath each strip. Place excess strips back in the foil pouch with the included desiccant pack and reseal. Store between 2°C and 8°C for up to one month.
Standard	The remaining reconstituted standard stock solution can be aliquoted into polypropylene vials and stored at -70°C for up to one month. Avoid repeated freeze-thaw cycles.
Avidin-HRP	Store opened reagent bottles at 2° - 8°C and use within 1 month
Assay Buffer B	
Wash Buffer (20X)	
Substrate Solution D	
Stop Solution	

**Health Hazard Warnings:**

1. Reagents that contain preservatives may be harmful if ingested, inhaled or absorbed through the skin. Refer to the MSDS online at BioLegend’s website for details ([www.biolegend.com/msds](http://www.biolegend.com/msds)).
2. Substrate Solution F is harmful if inhaled or ingested. Avoid skin, eye and clothing contact.
3. To reduce the likelihood of blood-borne transmission of infectious agents,

## LEGEND MAX™ Human CD26 (DPP4) ELISA Kit

handle all serum, plasma and other biological fluids in accordance with NCCLS regulations.

4. Stop Solution contains strong acid. *Wear eye, hand, and face protection.*
5. Before disposing of the plate, rinse it with an excess amount of tap water.

### Specimen Collection and Handling:

Specimens should be clear and non-hemolyzed. If possible, unknown samples should be run at a number of dilutions to determine the optimal dilution factor that will ensure accurate quantitation.

**Cell Culture Supernatant:** If necessary, centrifuge all samples to remove debris prior to analysis. It is recommended that samples be stored at  $< -70^{\circ}\text{C}$ . Avoid repeated freeze-thaw cycles.

**Serum:** Use a serum separator tube and allow clotting for at least 30 minutes, then centrifuge for 10 minutes at  $1,000 \times g$ . Remove serum layer and assay immediately or store serum samples at  $< -70^{\circ}\text{C}$ . Avoid repeated freeze-thaw cycles.

**Plasma:** Collect blood samples in citrate, heparin or EDTA containing tubes. Centrifuge for 10 minutes at  $1,000 \times g$  within 30 minutes of collection. Assay immediately or store plasma samples at  $< -70^{\circ}\text{C}$ . Avoid repeated freeze-thaw cycles.

**Saliva:** Collect saliva in a tube and centrifuge for 10 minutes at  $1,000 \times g$ . Collect the liquid layer, assay immediately or store at  $< -70^{\circ}\text{C}$ . Avoid repeated freeze-thaw cycles.

**Urine:** Collect urine in a tube and centrifuge for 10 minutes at  $1,000 \times g$  to remove particulate matter. Assay immediately or store at  $< -70^{\circ}\text{C}$ . Avoid repeated freeze-thaw cycles.

### Reagent and Sample Preparation:

Note: All reagents should be diluted immediately prior to use.

1. Dilute the 20X Wash Buffer to 1X with deionized water. For example, make 1 liter of 1X Wash Buffer by adding 50 mL of 20X Wash Buffer to 950 mL of deionized water. If crystals have formed in the 20X Wash Buffer, bring to room temperature and vortex until dissolved.
2. Reconstitute the lyophilized Human CD26 (DPP4) Lyophilized Standard by adding the volume of Assay Buffer B to make the 100 ng/mL standard stock solution (Refer to LEGEND MAX Kit Lot-Specific Certificate of Analysis/ LEGEND MAX Kit Protocol). Allow the reconstituted standard to sit at room temperature for 15-20 minutes, then briefly vortex to mix completely.
3. In general, 800-folds dilution in Assay Buffer B is recommended for serum and plasma samples. 16-folds dilution and 2-folds dilution are recommended for saliva and urine samples, respectively. Cell culture supernatant should be tested initially without any dilution. Samples can

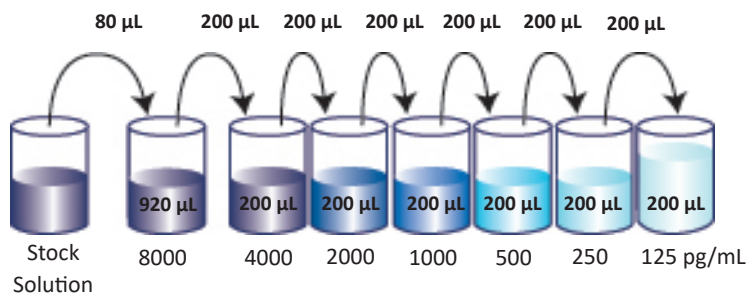
## LEGEND MAX™ Human CD26 (DPP4) ELISA Kit

be diluted further in Assay Buffer B to fit within the range of the assay as determined by the end user.

### Assay Procedure:

Note: Do not mix reagents from different kits or lots. Reagents and/or antibodies from different manufacturers should not be used with this kit.

1. Bring all reagents to room temperature prior to use. It is strongly recommended that all standards and samples be run in duplicate or triplicate. A standard curve is required for each assay.
2. If not all microplate strips will be used, remove the excess strips by pressing up from underneath each strip. Place excess strips back in the foil pouch with the included desiccant pack and reseal.
3. Prepare 1000  $\mu\text{L}$  of the 8000  $\text{pg/mL}$  top standard by adding 80  $\mu\text{L}$  of the 100  $\text{ng/mL}$  standard stock solution into 920  $\mu\text{L}$  Assay Buffer B. Perform six two-fold serial dilutions of the 8000  $\text{pg/mL}$  top standard in separate tubes using Assay Buffer B as the diluent. Thus, the hCD26 (DPP4) standard concentrations in the tubes are 8000  $\text{pg/mL}$ , 4000  $\text{pg/mL}$ , 2000  $\text{pg/mL}$ , 1000  $\text{pg/mL}$ , 500  $\text{pg/mL}$ , 250  $\text{pg/mL}$  and 125  $\text{pg/mL}$ , respectively. Assay Buffer B serves as the zero standard (0  $\text{pg/mL}$ ).



4. Wash the plate 4 times with at least 300  $\mu\text{L}$  of 1X Wash Buffer per well and blot any residual buffer by firmly tapping the plate upside down on absorbent paper. All subsequent washes should be performed similarly.
5. Add 50  $\mu\text{L}$  of Assay Buffer B to each well that will contain either standard dilutions or samples. Then add 50  $\mu\text{L}$  of standard dilutions or samples to the appropriate wells.
6. Seal the plate with a Plate Sealer included in the kit and incubate the plate for 2 hours at room temperature with shaking.
7. Discard the contents of the plate into a sink, then wash the plate 4 times with 1X Wash Buffer as in step 4.
8. Add 100  $\mu\text{L}$  of Human CD26 (DPP4) Detection Antibody solution to each well,

## LEGEND MAX™ Human CD26 (DPP4) ELISA Kit

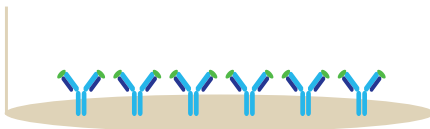
seal the plate and incubate at room temperature for 1 hour while shaking.

9. Discard the contents of the plate into a sink, then wash the plate 4 times with 1X Wash Buffer as in step 4.
10. Add 100  $\mu$ L of Avidin-HRP solution to each well, seal the plate and incubate at room temperature for 30 minutes while shaking.
11. Discard the contents of the plate into a sink, then wash the plate 5 times with 1X Wash Buffer as in step 4. For this final wash, soak wells in 1X Wash Buffer for 30 seconds to 1 minute for each wash. This will help minimize background.
12. Add 100  $\mu$ L of Substrate Solution D to each well and incubate for 20 minutes in the dark. Wells containing Human CD26 (DPP4) should turn blue in color with an intensity proportional to its concentration. It is not necessary to seal the plate during this step.
13. Stop the reaction by adding 100  $\mu$ L of Stop Solution to each well. The solution color should change from blue to yellow.
14. Read absorbance at 450 nm within 30 minutes. If the reader is capable of reading at 570 nm, the absorbance at 570 nm can be subtracted from the absorbance at 450 nm.

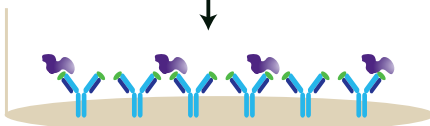


## Assay Procedure Summary

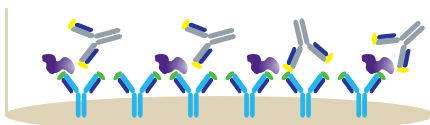
1. Wash 4 times.  
Add 50  $\mu$ L Assay Buffer B to standard wells and sample wells



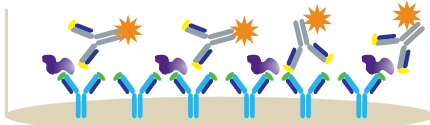
2. Add 50  $\mu$ L of standard or sample, incubate 2 hr, RT, shaking



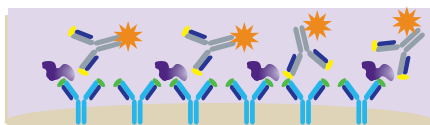
3. Wash 4 times  
Add 100  $\mu$ L of Human CD26 (DPP4) Detection Antibody solution.  
Incubate 1 hr, RT, shaking



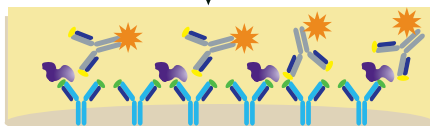
4. Wash 4 times  
Add 100  $\mu$ L Avidin-HRP solution.  
Incubate 30 min, RT, shaking



5. Wash 5 times  
Add 100  $\mu$ L Substrate Solution D  
Incubate 20 min, RT, in the dark



6. Add 100  $\mu$ L Stop Solution



7. Read absorbance at 450 nm and 570 nm

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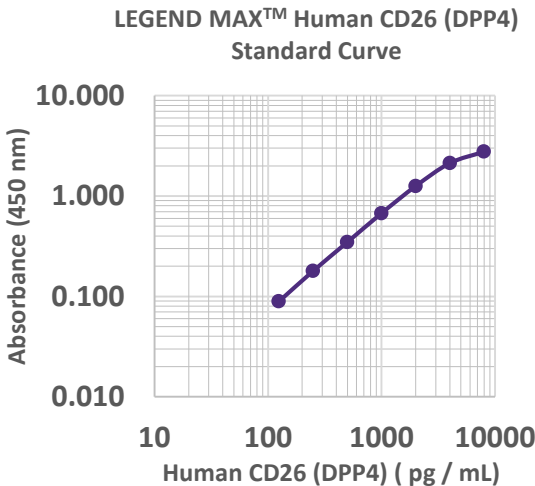
## Calculation of Results:

The data can be best calculated with computer-based curve-fitting software using a 5- or 4-parameter logistics curve-fitting algorithm. If an appropriate software is not available, use log-log graph paper to determine sample concentrations. Determine the mean absorbance for each set of duplicate or triplicate standards, controls, and samples. Plot the standard curve on log-log graph paper with analyte concentration on the X-axis and absorbance on the Y-axis. Draw a best fit line through the standard points. To determine the unknown analyte concentrations, find the mean absorbance value of the unknown concentration on the Y-axis and draw a horizontal line to the standard curve. At the point of intersection, draw a vertical line to the X-axis and read the analyte concentration.

If samples were diluted, multiply the concentration by the appropriate dilution factor. If a test sample's absorbance value falls outside the linear portion of the standard curve, the test sample needs to be re-analyzed at a higher (or lower) dilution as appropriate.

## Typical Data:

This standard curve was generated at BioLegend for demonstration purposes only. A standard curve must be run with each assay.



## Performance Characteristics:

**Specificity:** This kit recognizes natural and recombinant Human CD26 (DPP4). No cross reactivity was observed when this kit was used to analyze the following recombinant proteins at 50 ng/mL.

Human	ECE1, ECE2, ACE, ACE2, FAP, CD10
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**Sensitivity:** The minimum detectable concentration of Human CD26 (DPP4) is  $21.3 \pm 4.5$  pg/mL (n=6).

**Recovery:** Recombinant Human CD26 (DPP4) at 3 different concentrations was spiked into human samples: Serum, Citrate Plasma, EDTA Plasma, and Heparin Plasma, Saliva, Urine, and cell culture supernatant. Sample recovery was then analyzed with the LEGEND MAX™ Human CD26 (DPP4) Kit.

Sample Type	N	% Recovery
Serum	3	96.0%
Citrate Plasma	3	101.3%
EDTA Plasma	3	98.6%
Heparin Plasma	3	93.6%
Pooled Saliva	1	97.0%
Pooled Urine	1	111.0%
Cell Culture Supernatant	1	74.0%

**Linearity:** Natural human Serum and Plasma samples were first diluted 100 folds. Saliva were first diluted 4 folds, and no initial dilution was done to either human urine or cell culture supernatant. Then, the samples were diluted 2 fold in serial to produce samples within the dynamic range of the kit. Samples were then assayed to determine the dilutional linearity.

Sample Type	N	% Linearity
Serum	3	106.0%
Citrate Plasma	3	103.6%
EDTA Plasma	3	104.0%
Heparin Plasma	3	105.3%
Pooled Saliva	1	104%
Pooled Urine	1	111%
Cell Culture Supernatant	1	106%

## LEGEND MAX™ Human CD26 (DPP4) ELISA Kit

**Intra-Assay Precision:** Two samples containing different human CD26 (DPP4) concentrations were tested on one plate with 12 replicates.

Concentration	Sample 1	Sample 2
Number of Replicates	12	12
Mean Concentration (pg/mL)	1710	447
Standard Deviation	21.8	7.1
%CV	1.3%	1.6%

**Inter-Assay Precision:** Two samples containing different human CD26 (DPP4) concentrations were tested in ten independent assays.

Concentration	Sample 1	Sample 2
Number of Assays	10	10
Mean Concentration (pg/mL)	1576	421
Standard Deviation	98.0	27.4
%CV	6.2%	6.5%

**Biological Samples:** Human Serum, Citrate Plasma, EDTA Plasma, Heparin Plasma, Saliva, Urine were assayed for natural CD26 (DPP4).

	Serum	Citrate Plasma	EDTA Plasma	Heparin Plasma	Saliva (Pooled Sample)	Urine (Pooled Sample)
N	5	5	4	6	1	1
Min (ng/mL)	416	386	402	517	NA	NA
Max (ng/mL)	860	562	645	661	NA	NA
Mean (ng/mL)	653	483	534	592	14.5	1.87

Human peripheral blood cells ( $1 \times 10^6$  cells/mL) were cultured in RPMI, 10% fetal bovine serum, 100 U/mL penicillin-streptomycin. Cells were cultured unstimulated or stimulated with 10  $\mu$ g/mL PHA for 72 hours. Cell culture supernatant was assayed for natural CD26 (DPP4).

	Unstimulated	Stimulated
pg/mL	150	540

## LEGEND MAX™ Human CD26 (DPP4) ELISA Kit

### Troubleshooting Guide:

Problem	Probable Cause	Solution
High Background	Background wells were contaminated	Avoid cross-well contamination by using the provided plate sealers. Use multichannel pipettes and change tips between pipetting samples and reagents.
	Insufficient washes	Increase number of washes. Increase soaking time between washes prior to addition of substrate solution.
	TMB Substrate Solution was contaminated	TMB Substrate Solution should be clear and colorless prior to addition to wells. Use a clean container prior to pipetting substrate solution into wells.
No or poor signal	Detection Antibody, Avidin-HRP or Substrate solution were NOT added	Rerun the assay and follow the protocol.
	Wrong reagent or reagents were added in wrong sequential order	
	Insufficient plate agitation	The plate should be agitated during all incubation steps using a plate shaker at a speed where solutions in wells are within constant motion without splashing.
	The wash buffer contains Sodium Azide (NaN <sub>3</sub> )	Avoid Sodium Azide contamination in the wash buffer as it inhibits HRP activity.
	Incubations were done at an inappropriate temperature, timing or without agitation	Rerun the assay and follow the protocol.
Low or poor standard curve signal	The standard was incorrectly reconstituted or diluted	Adjust the calculations and follow the protocol.
	Standard was inappropriately stored	Store the reconstituted standard stock solution in polypropylene vials at -70°C. Avoid repeated freeze-thaw cycles.
	Reagents added to wells with incorrect concentrations	Check for pipetting errors and the correct reagent volume.

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Problem	Probable Cause	Solution
Signal is high, standard curves have saturated signal	Standard reconstituted with less volume than required	Reconstitute new lyophilized standard with the correct volume of solution recommended in the protocol.
	Standards/samples, detection antibody, Avidin-HRP or substrate solution were incubated for too long	Rerun the assay and follow the protocol.
Sample readings are out of range	Samples contain no or below detectable levels of the analyte	If samples are below detectable levels, it may be possible to use a larger sample volume. Contact technical support for appropriate protocol modifications.
	Samples contain analyte concentrations greater than highest standard point	Samples may require dilution and analysis.
High variation in samples and/or standards	Multichannel pipette errors	Confirm that pipette calibrations are accurate.
	Plate washing was not adequate or uniform	Ensure pipette tips are tightly secured. Ensure uniformity in all wash steps.
	Non-homogenous samples	Thoroughly mix samples before assaying.
	Samples may have high particulate matter	Remove particulate matter by centrifugation.
	Cross-well contamination	Do not reuse plate sealers.  Always change tips for reagent additions. Ensure that pipette tips do not touch the reagents on the plate.

**ELISA Plate Template**

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



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