ab204722

Dipeptidyl peptidase IV (DPP4) Activity Assay Kit (Fluorometric)

Instructions for Use

For rapid, sensitive and accurate detection of Dipeptidyl peptidase IV (DPP4) activity.

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

Version 7a, Last Updated 6 June 2025

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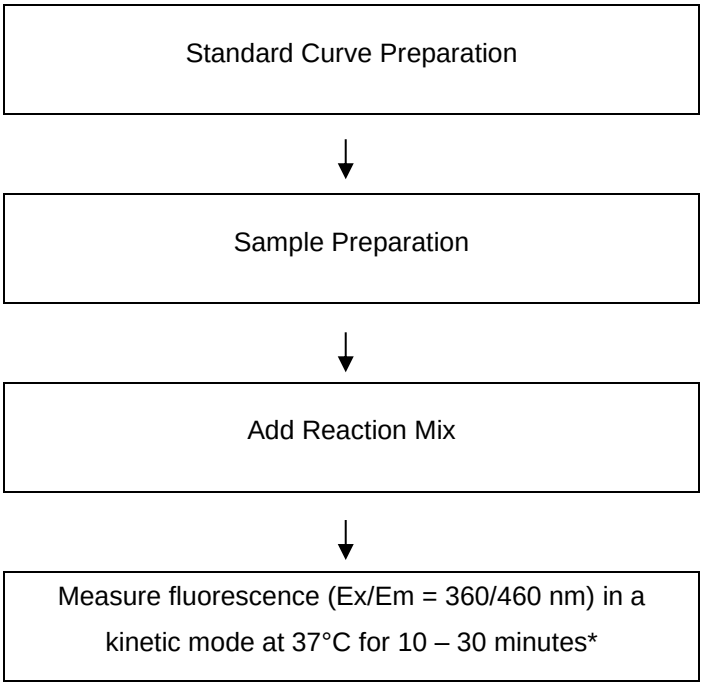
1. BACKGROUND

Dipeptidyl peptidase IV (DPP4) Activity Assay Kit (Fluorometric) (ab204722) is an assay where DPP4 cleaves a substrate to release a quenched fluorescent group, AMC (7-Amino-4-Methyl Coumarin), which can be easily detected at Ex/Em = 360/460 nm. This assay is rapid, simple, sensitive, and reliable, as well as, suitable for high throughput activity screening of DPP4. This kit detects DPP4 activity as low as 3 µU per well.

Dipeptidyl peptidase-4 (DPP4), also known as adenosine deaminase complexing protein 2 or CD26 (cluster of differentiation 26) is a protein that, in humans, is encoded by the DPP4 gene. The substrates of CD26/DPP4 are proline (or alanine) containing peptides and include growth factors, chemokines, neuropeptides, and vasoactive peptides. DPP4 plays a major role in glucose metabolism. It is responsible for the degradation of incretins such as GLP-1 and hence its inhibition by drugs such as Sitagliptin have been used for treatment of diabetes mellitus type 2. DPP4 also appears to work as a suppressor in the development of cancer and tumors.

2. ASSAY SUMMARY



**For kinetic mode detection, incubation time given in this summary is for guidance only.*

3. PRECAUTIONS

Please read these instructions carefully prior to beginning the assay.

All kit components have been formulated and quality control tested to function successfully as a kit. Modifications to the kit components or procedures may result in loss of performance.

4. STORAGE AND STABILITY

Store kit at -20ºC in the dark immediately upon receipt. Kit has a storage time of 1 year from receipt, providing components have not been reconstituted.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in the Materials Supplied section.

Aliquot components in working volumes before storing at the recommended temperature.

5. LIMITATIONS

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

6. MATERIALS SUPPLIED

Item	Amount	Storage Condition (Before Preparation)	Storage Condition (After Preparation)
DPP4 Assay Buffer	25 mL	-20°C	-20°C
DPP4 Substrate	200 µL	-20°C	-20°C
DPP4 Positive Control	20 µL	-20°C	-20°C
AMC Standard	100 µL	-20°C	-20°C
DPP4 Inhibitor	1 mL	-20°C	-20°C

7. MATERIALS REQUIRED, NOT SUPPLIED

These materials are not included in the kit, but will be required to successfully perform this assay:

- MilliQ water or other type of double distilled water (ddH_2O)
- PBS
- Microcentrifuge
- Pipettes and pipette tips
- Fluorescent microplate reader – equipped with filter Ex/Em =360/460 nm
- White 96-well plate
- Heat block or water bath
- Dounce homogenizer (if using tissue)

8. TECHNICAL HINTS

- **This kit is sold based on number of tests. A 'test' simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.**
- Selected components in this kit are supplied in surplus amount to account for additional dilutions, evaporation, or instrumentation settings where higher volumes are required. They should be disposed of in accordance with established safety procedures.
- Keep enzymes, heat labile components and samples on ice during the assay.
- Make sure all buffers and solutions are at room temperature before starting the experiment.
- Samples generating values higher than the highest standard should be further diluted in the appropriate sample dilution buffers.
- Avoid foaming or bubbles when mixing or reconstituting components.
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions.
- Ensure plates are properly sealed or covered during incubation steps.
- Make sure you have the right type of plate for your detection method of choice.
- Make sure the heat block/water bath and microplate reader are switched on.

9. REAGENT PREPARATION

- Briefly centrifuge small vials at low speed prior to opening.

9.1 **DPP4 Assay Buffer:**

Ready to use as supplied. Equilibrate to room temperature before use. Store at -20°C.

9.2 **DPP4 Substrate:**

Ready to use as supplied. Aliquot substrate so that you have enough volume to perform the desired number of assays. Store at -20°C. Keep on ice while in use.

9.3 **DPP4 Positive Control:**

Ready to use as supplied. Aliquot positive control so that you have enough volume to perform the desired number of assays. Store at -20°C. Keep on ice while in use.

9.4 **AMC Standard:**

Ready to use as supplied. Aliquot standard so that you have enough volume to perform the desired number of assays. Store at -20°C. Keep on ice while in use.

9.5 **DPP4 Inhibitor:**

Ready to use as supplied. Aliquot inhibitor so that you have enough volume to perform the desired number of assays. Store at -20°C. Keep on ice while in use.

10. STANDARD PREPARATION

- Always prepare a fresh set of standards for every use.
- Diluted standard solution is unstable and cannot be stored for future use.

10.1 Prepare 1 mL of 10 µM AMC standard by diluting 10 µL of the provided 1 mM AMC standard with 990 µL of dH_2O.

10.2 Using 10 µM AMC standard, prepare standard curve dilution as described in the table in a microplate.

Standard #	Volume of Standard (µL)	Assay Buffer (µL)	Final volume standard in well (µL)	Amount of substance (pmol/well)
1	0	300	100	0
2	6	294	100	20
3	12	288	100	40
4	18	282	100	60
5	24	276	100	80
6	30	270	100	100

Each dilution has enough amount of standard to set up duplicate readings (2 x 100 µL).

11. SAMPLE PREPARATION

General Sample information:

- We recommend performing several dilutions of your sample to ensure the readings are within the standard value range.
- We recommend that you use fresh samples. If you cannot perform the assay at the same time, we suggest that you complete the Sample Preparation step before storing the samples. Alternatively, if that is not possible, we suggest that you snap freeze cells or tissue in liquid nitrogen upon extraction and store the samples immediately at -80°C. When you are ready to test your samples, thaw them on ice. Be aware however that this might affect the stability of your samples and the readings can be lower than expected.

11.1 **Cell (adherent or suspension) samples:**

11.1.1 Harvest the amount of cells necessary for each assay (initial recommendation = 2 x 10^6 cells).

11.1.2 Homogenize cells in 4 volumes of DPP4 Assay Buffer quickly by pipetting up and down a few times.

11.1.3 Centrifuge sample for 10 minutes at 4°C at top speed in a cold microcentrifuge to remove any insoluble material.

11.1.4 Collect supernatant and transfer to a clean pre-chilled tube and store on ice. Use lysates immediately to assay DPP4 activity.

11.2 **Tissue samples:**

11.2.1 Harvest the amount of tissue necessary for each assay (initial recommendation = 10 mg).

11.2.2 Homogenize tissue in 4 volumes of DPP4 Assay Buffer with a Dounce homogenizer sitting on ice, with 10 – 15 passes.

11.2.3 Centrifuge samples for 10 minutes at 4°C at top speed using a cold microcentrifuge to remove any insoluble material.

11.2.4 Collect supernatant and transfer to a clean pre-chilled tube and store on ice. Use lysates immediately to assay DPP4 activity.

11.3 **Serum:**

Serum can be tested directly by adding sample to the microplate wells.

However, to find the optimal values and ensure your readings will fall within the standard values, we recommend performing several dilutions of the sample.

NOTE: *We suggest using different volumes of sample to ensure readings are within the Standard Curve range.*

12. ASSAY PROCEDURE and DETECTION

- **Equilibrate all materials and prepared reagents to room temperature prior to use.**
- **It is recommended to assay all standards, controls and samples in duplicate.**
- **Prepare all reagents, working standards, and samples as directed in the previous sections.**

12.1 **Set up Reaction wells:**

- Standard wells = 100 µL standard dilutions.
- Sample wells = 2 – 50 µL samples (adjust volume to 50 µL/well with DPP4 Assay Buffer). For each sample, use a replicate as Background control, see section 12.2.
- Positive control = 1 – 2 µL DPP4 Positive control (adjust volume to 50 µL/well with DPP4 Assay Buffer).

12.2 **Background wells:**

- For every sample, use a replicate as background control well. Add10 µL DPP4 Inhibitor to the background control sample well. For the other sample replicate add 10µl DPP4 Assay Buffer. Mix well and incubate for 10 minutes at 37°C.

12.3 **Reaction Mix:**

Prepare 40 µL of Reaction Mix for each reaction:

Component	Reaction Mix (µL)
DPP4 Assay Buffer	**38**
DPP4 Substrate	**2**

Mix enough reagents for the number of assays (samples and background controls) to be performed. Prepare a master mix of the Reaction Mix to ensure consistency. We recommend the following calculation: X µL component x (Number samples +1)

12.4 Add 40 µL of Reaction Mix into each sample and Background control sample wells. Do not add reaction mix to the Standard Curve wells. Mix well.

12.5 Measure output on a fluorescent microplate reader at Ex/Em = 360/460 nm in a kinetic mode for at least 30 minutes at 37°C protected from light.

NOTE: *Sample incubation time can vary depending on Dipeptidyl peptidase IV (DPP4) activity in the samples. We recommend measuring fluorescence in kinetic mode and then choosing two time points (T_1 and T_2) during the linear range.*

RFU value at T_2 should not exceed the highest RFU in the standard curve. For standard curve, do not subtract RFU_1 from RFU_2 reading. Standard curve can be read in end-point mode.

13. CALCULATIONS

- Samples producing signals greater than that of the highest standard should be further diluted in appropriate buffer and reanalyzed, then multiplying the concentration found by the appropriate dilution factor.
- For statistical reasons, we recommend each sample should be assayed with a minimum of two replicates (duplicates).

13.1 Average the duplicate reading for each standard and sample.

13.2 If the sample background control is significant, then subtract the sample background control from sample reading.

13.3 Subtract the mean absorbance value of the blank (Standard #1) from all standard and sample readings. This is the corrected absorbance.

13.4 Plot the corrected absorbance values for each standard as a function of the final concentration of AMC.

13.5 Draw the best smooth curve through these points to construct the standard curve. Calculate the trend line equation based on your standard curve data (use the equation that provides the most accurate fit).

13.6 Activity of DPP4 is calculated as:

$$\mathbf{\Delta RFU_{360/460nm} = (RFU_2 - RFU_{2BG}) - (RFU_1 - RFU_{1BG})}$$

Where:

RFU_1 is the sample reading at time T1.

RFU_{1BG} is the background control sample at time T1.

RFU_2 is the sample reading at time T2.

RFU_{2BG} is the background control sample at time T2.

13.7 Use the $\Delta RFU_{360/460nm}$ to obtain B pmol of AMC generated by DPP4 during the reaction time ($\Delta T = T_2 - T_1$).

13.8 Activity of DPP4 in the test samples is calculated as:

$$Activity = \frac{B}{(T2 - T1)\ x\ V} * D = pmol/min/mL = \mu U/mL$$

Where:

B = Amount of AMC from Standard Curve (pmol).

T_1 = Time of the first reading (minutes).

T_2 = Time of the second reading (minutes).

V = Original sample volume added into the reaction well (mL).

D = Sample dilution factor.

Unit Definition:

1 Unit DPP4 activity = amount of Dipeptidyl peptidase IV (DPP4) that hydrolyzes the DPP4 Substrate to yield 1.0 µmol of AMC per minute at 37°C.

14.TYPICAL DATA

TYPICAL STANDARD CURVE – Data provided for **demonstration purposes only**. A new standard curve must be generated for each assay performed.

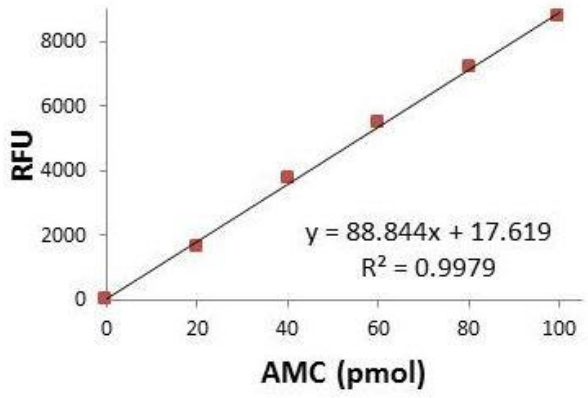


Figure 1. Typical AMC Standard calibration curve.

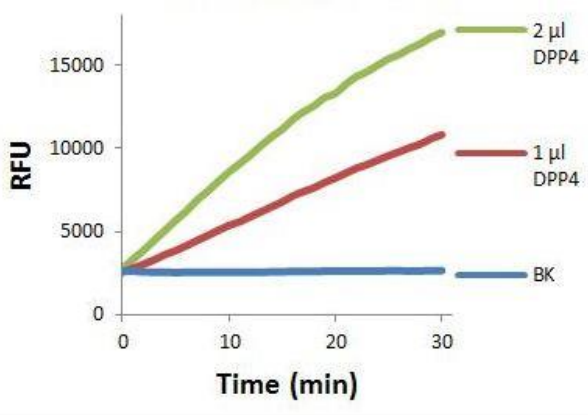


Figure 2. Dipeptidyl peptidase IV (DPP4) Positive Control.

15. QUICK ASSAY PROCEDURE

***NOTE**: This procedure is provided as a quick reference for experienced users. Follow the detailed procedure when performing the assay for the first time.*

- Prepare standards and prepare enzyme mix (aliquot if necessary); get equipment ready.
- Prepare appropriate standard curve.
- Prepare samples in duplicate (find optimal dilutions to fit standard curve readings).
- Set up plate for standard (100 µL), samples (50 µL) and background wells (50 µL).
- Incubate background control wells for 10 minutes at 37°C.
- Prepare Dipeptidyl peptidase IV (DPP4) Reaction Mix (Number samples + 1).

Component	Reaction Mix (µL)
DPP4 Assay Buffer	**38**
DPP4 Substrate	**2**

- Add 40 µL of Dipeptidyl peptidase IV (DPP4) Reaction Mix to the sample wells.
- Incubate plate at 37°C during 30 minutes and read fluorescence at Ex/Em= 360/460 nm in a kinetic mode.

16. TROUBLESHOOTING

Problem	Cause	Solution
Assay not working	Use of ice-cold buffer	Buffers must be at room temperature
	Plate read at incorrect wavelength	Check the wavelength and filter settings of instrument
	Use of a different 96-well plate	Colorimetric: Clear plates Fluorometric: black wells/clear bottom plate
Sample with erratic readings	Samples not deproteinized (if indicated on protocol)	Use PCA precipitation protocol for deproteinization
	Cells/tissue samples not homogenized completely	Use Dounce homogenizer, increase number of strokes
	Samples used after multiple free/ thaw cycles	Aliquot and freeze samples if needed to use multiple times
	Use of old or inappropriately stored samples	Use fresh samples or store at - 80°C (after snap freeze in liquid nitrogen) till use
	Presence of interfering substance in the sample	Check protocol for interfering substances; deproteinize samples
Lower/ Higher readings in samples and Standards	Improperly thawed components	Thaw all components completely and mix gently before use
	Allowing reagents to sit for extended times on ice	Always thaw and prepare fresh reaction mix before use
	Incorrect incubation times or temperatures	Verify correct incubation times and temperatures in protocol

Problem	Cause	Solution
Standard readings do not follow a linear pattern	Pipetting errors in standard or reaction mix	Avoid pipetting small volumes (< 5 µL) and prepare a master mix whenever possible
	Air bubbles formed in well	Pipette gently against the wall of the tubes
	Standard stock is at incorrect concentration	Always refer to dilutions on protocol
Unanticipated results	Measured at incorrect wavelength	Check equipment and filter setting
	Samples contain interfering substances	Troubleshoot if it interferes with the kit
	Sample readings above/ below the linear range	Concentrate/ Dilute sample so it is within the linear range

17.INTERFERENCES

These chemicals or biological materials will cause interferences in this assay causing compromised results or complete failure:

- RIPA buffer – it contains SDS which can destroy/decrease the activity of the enzyme.

18.FAQ

19.NOTES

Technical Support

For all technical or commercial enquiries please go to:

www.abcam.com/contactus

www.abcam.cn/contactus (China)

www.abcam.co.jp/contactus (Japan)