Description

The KLK2 Fluorogenic Assay Kit is designed to measure KLK2 (Kallikrein-2) protease activity for screening and profiling applications. The assay kit comes in a convenient 96-well format, with enough recombinant KLK2 (amino acids 25-261), fluorogenic AMC-based peptide substrate, and assay buffer for 100 enzyme reactions.

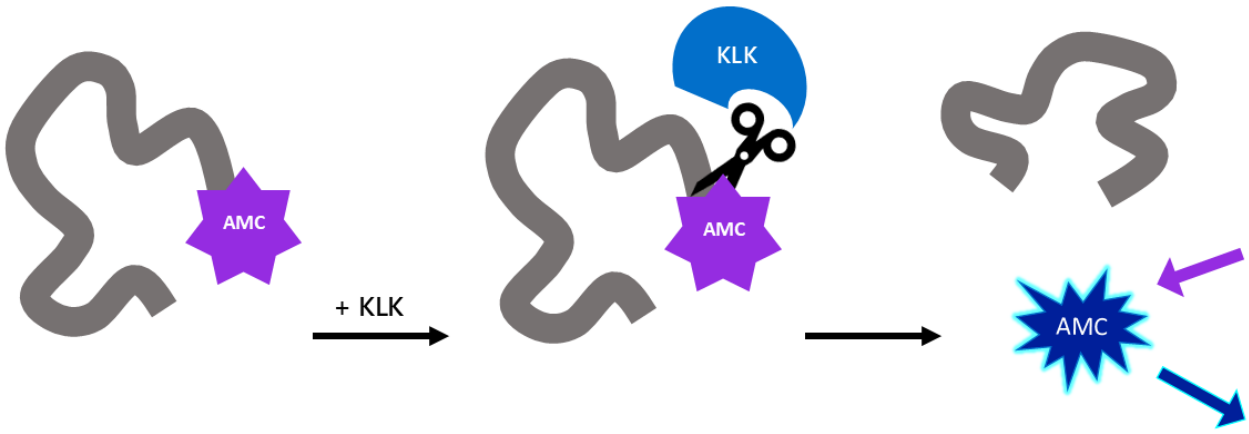

Figure 1: Illustration of the assay principle.
A peptide substrate conjugated to AMC is incubated with KLK2. The conjugated AMC displays only weak fluorescence. Upon proteolysis, AMC is no longer quenched and exhibits strong blue fluorescence. The increase in fluorescence is proportional to the KLK2 activity.

Background

Kallikrein-2 (KLK2) is a trypsin-like serine protease. This protein is a member of the glandular kallikrein family and is critical in the enzymatic process as it selectively cleaves the Met-Lys and Arg-Ser bonds in kininogen, thereby releasing Lys-bradykinin. This proteolytic activity contributes to the production of bradykinin, a bioactive peptide with multiple roles in regulating blood pressure, inflammation, and vascular permeability. It is expressed by prostate epithelial cells and is regulated by the androgen receptor (AR). Its expression in prostate adenocarcinoma is nearly ubiquitous, and high expression levels are maintained in metastatic castration-resistant PC (mCRPC). While traditionally seen as secreted only, it is also present on the cancer cell surface. Its pattern of expression makes it an ideal target in the treatment of mCRPC using novel therapies, such as radioligands and immune-based therapies (like bispecific T cell engagers). Pasritamig is a clinical-stage human bispecific antibody that simultaneously binds to CD3 on T cells and cell surface KLK2 on tumor cells, enhancing the cytotoxicity towards KLK2-expressing tumor cells and it is currently under clinical trial. A deeper understanding of the role of membrane-bound or intracellular KLK2 and investigation into therapies will bring benefit to prostate cancer patients.

Applications

Enzyme kinetics studies and screening small molecule inhibitors for drug discovery and high-throughput (HTS) applications.

Supplied Materials

Catalog #	Name	Amount	Storage
83709-KC2.5^{+}	Kallikrein-2(KLK2), Lyophilized	2.5 µg	-80°C
84003-KC5	PR Substrate 4	5 µl	-80°C
84004-KC10	PR-03 Assay Buffer	10 ml	-80°C
79685	96-well black microplate	1	Room Temp

Materials Required but Not Supplied

- Adjustable micropipettor and sterile tips
- Fluorimeter capable of excitation at λ=380 nm and detection at λ=460 nm

Stability

This assay kit will perform optimally for up to **6 months** from date of receipt when the materials are stored as directed.

Safety

This product is for research purposes only and not for human or therapeutic use. This product should be considered hazardous and is harmful by inhalation, in contact with skin, eyes, clothing, and if swallowed. If contact occurs, wash thoroughly.

Contraindications

- The final concentration of DMSO in the assay should not exceed 1%.
- Compounds that are fluorescent may interfere with the results, depending on their spectral excitation and emission properties.
- It is recommended that the compound alone is tested to determine any potential interference of the compound with the assay results.

Assay Protocol

- All samples and controls should be performed in duplicates.
- The assay should include “Negative Control”, “Positive Control,” and a “Test Inhibitor” conditions.
- We recommend maintaining the diluted protein on ice during use.
- For detailed information on protein handling please refer to Protein FAQs (bpsbioscience.com).
- We recommend using Gabexate Mesylate (BPs Bioscience #84177) as internal control. If not running a dose response curve for the control inhibitor, we recommend running the control inhibitor at 0.1X, 1X and 10X the IC_{50} value shown in the validation data below.

1. Resuspend KLK2 with 5 µl of distilled water to get a concentration of 0.5 mg/ml.

2. Dilute KLK2 1000-fold to 0.5 ng/µl with **PR-03 Assay Buffer** (you need 20 µl/well).

3. Add 20 µl of **diluted KLK2** to all wells except to the “Negative Control” wells.

4. Add 20 µl of **PR-03 Assay Buffer** to the “Negative Control” wells.

5. Prepare the **Test inhibitor** (5 µl/well): for a titration, prepare serial dilutions at concentrations 10-fold higher than the desired final concentrations. The final volume of the reaction is 50 µl.

   5.1 If the Test Inhibitor is water-soluble, prepare serial dilutions in the PR-03 Assay Buffer at concentrations 10-fold higher than the desired final concentrations.

   For positive and negative controls, use PR-03 Assay Buffer (Diluent Solution)

   OR

   5.2 If the Test inhibitor is soluble in DMSO, prepare the test inhibitor in 100% DMSO at a concentration 100-fold higher than the highest desired final concentration, then dilute the inhibitor 10-fold in PR-03 Assay Buffer to prepare the highest concentration of the serial dilutions. The concentration of DMSO is now 10%.

   Using PR-03 Assay Buffer containing 10% DMSO to keep the concentration of DMSO constant, prepare serial dilutions of the Test Inhibitor at 10-fold the desired final concentrations.

   For positive and negative controls, prepare 10% DMSO in PR-03 Assay Buffer (vol/vol) so that all wells contain the same amount of DMSO (Diluent Solution).

   Note: The final concentration of DMSO in the assay should not exceed 1%.

6. Add 5 µl of **Test inhibitor** to each well designated "Test Inhibitor".

7. Add 5 µl of **Diluent Solution** to the "Positive Control" and "Negative Control" wells.

8. Preincubate the Test inhibitor with the diluted KLK2 for 30 minutes at Room Temperature (RT) with gentle agitation.

9. Dilute 500-fold the **PR Substrate 4** with PR-03 Assay Buffer (25 µl/well).

10. Initiate the reaction by adding 25 µl of **diluted PR Substrate 4** to all wells.

Protect your samples from direct exposure to light.

11. Incubate at RT for 30 minutes.

Component	Negative Control	Positive Control	Test Inhibitor
PR-03 Assay Buffer	20 µl	-	-
Test Inhibitor	-	-	5 µl
Diluent Solution	5 µl	5 µl	-
Diluted KLK2 (0.5 ng/µl)	-	20 µl	20 µl
Pre-incubate 30 minutes at Room Temperature			
Diluted PR Substrate 4	25 µl	25 µl	25 µl
Total	**50 µl**	**50 µl**	**50 µl**

12. Read the fluorescence intensity of the samples (λexcitation = 380 nm; λemission = 460 nm, bandwidth = 10) in a fluorescence reader.

Example Results

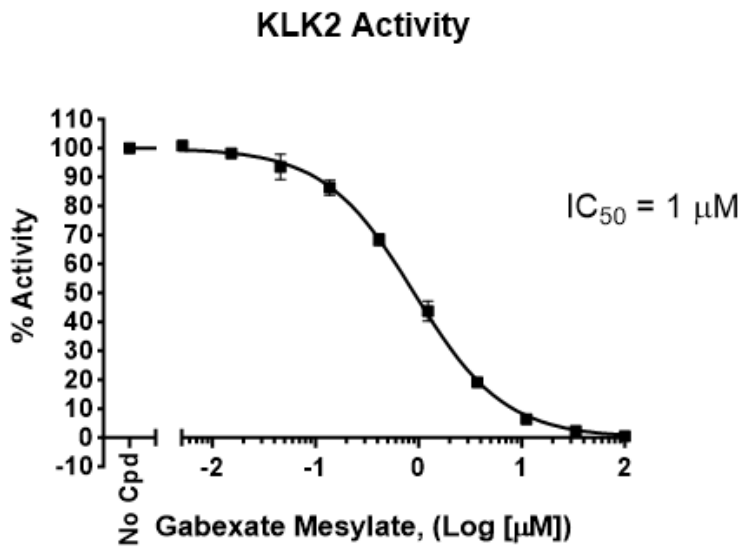

Figure 2. KLK2 activity is inhibited by Gabexate Mesylate.
KLK2 activity was measured in the presence of increasing concentrations of Gabexate Mesylate (#84177). Results are expressed as percentage of activity relative to the positive control (measured in the absence of inhibitor and set at 100%).

Data shown is representative.

Troubleshooting Guide
Visit bpsbioscience.com/assay-kits-faq for detailed troubleshooting instructions. For lot-specific information and all other questions, please visit https://bpsbioscience.com/contact.

References

Petraki C. D., *et al.,* 2006 *Prostate Cancer and Prostatic Diseases*. 9(2): 122–128.
Shang Z, *et al*., 2014. *Tumour Biol*. 35(3):1881-90.
Fei S., *et al.,* 2025. *Clinical Cancer Research*, epub July 8, 2025.
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