

Description

The KLK5 Fluorogenic Assay Kit is designed to measure KLK5 (Kallikrein-5) protease activity for screening and profiling applications. The assay kit comes in a convenient 96-well format, with enough recombinant KLK5 (amino acids 67-293), fluorogenic AMC-based peptide substrate, and assay buffer for 100 enzyme reactions.

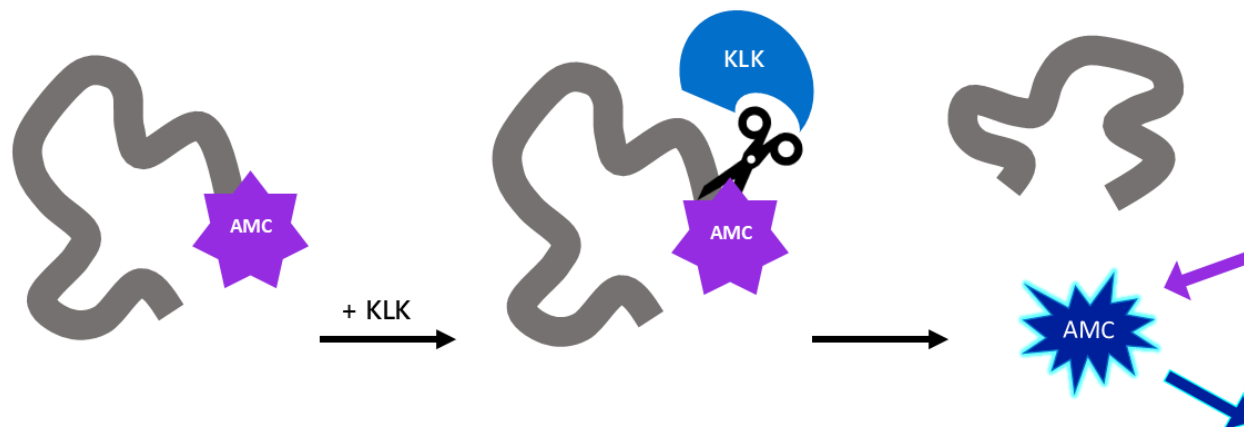


Figure 1: Illustration of the assay principle.

A peptide substrate conjugated to AMC is incubated with KLK5. 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 KLK5 activity.

Background

Kallikrein-5 (KLK5), previously known as SCTE (stratum corneum tryptic enzyme) is a serine protease, mostly expressed in the epidermis. It plays a potential role in desquamation, suggesting an involvement in the intricate processes of skin shedding and exfoliation. Its potential connection to desquamation implies a functional role in regulating the removal of dead skin cells, a crucial aspect of skin homeostasis. Its levels are regulated by estrogens and progestins, and this protein has been linked to cancer. KLK5 is regulated by ions link zinc and can be inhibited by serpins. The development of inhibitors selective for KLK5, like Kallikrein 5-IN-2, can potentially provide relief to patients with skin inflammation and itching.

Applications

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

Supplied Materials

Catalog #	Name	Amount	Storage
84079-KC6 ⁺	Kallikrein-5 (KLK5), Lyophilized	6 µg	-80°C
84077-KC5	PR Substrate 5	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 $\lambda=380$ nm and detection at $\lambda=460$ 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 \(bpsbioscience.com\)](https://www.bpsbioscience.com/protein-faqs).
- We recommend using Kallikrein 5-IN-2 (BPS Bioscience #83687) 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 IC₅₀ value shown in the validation data below.

1. Resuspend **KLK5** with 12 μ l of distilled water to get a concentration of 0.5 mg/ml.
2. Dilute 200-fold the KLK5 to 2.5 ng/ μ l with **PR-03 Assay Buffer** (you need 20 μ l/well).
3. Add 20 μ l of **diluted KLK5** to all wells except “Negative Control” wells.
4. Add 20 μ l of **PR-03 Assay Buffer** to the “Negative Control” wells.
5. Prepare the **Test inhibitor** (5 μ l/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 μ l.

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 KLK5 for 30 minutes at Room Temperature (RT) with gentle agitation.
9. Dilute 500-fold the **PR Substrate 5** with PR-03 Assay Buffer (25 µl/ well).
10. Initiate the reaction by adding 25 µl of **diluted PR Substrate 5** 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 KLK5 (2.5 ng/µl)	-	20 µl	20 µl
Pre-incubate 30 minutes at Room Temperature			
Diluted PR Substrate 5	25 µl	25 µl	25 µl
Total	50 µl	50 µl	50 µl

12. Read the fluorescence intensity of the samples ($\lambda_{\text{excitation}} = 380 \text{ nm}$; $\lambda_{\text{emission}} = 460 \text{ nm}$, bandwidth = 10) in a fluorescence reader.

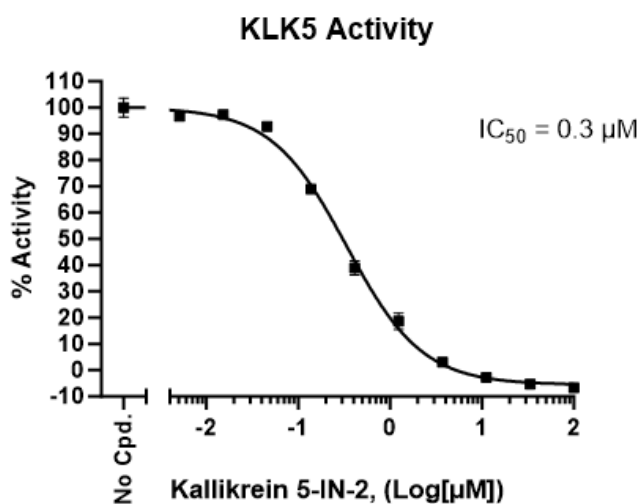
Example Results:

Figure 2. KLK5 activity is inhibited by Kallikrein 5-IN-2.

KLK5 activity was measured in the presence of increasing concentrations of Kallikrein 5-IN-2. 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

Li Y.-C., et al., 2022 *Biomedicines* 10 (2): 474.

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