3050 Spruce Street, St. Louis, MO 63103 USA
Tel: (800) 521-8956 (314) 771-5765 Fax: (800) 325-5052 (314) 771-5757
email: techservice@sial.com sigma-aldrich.com

Product Information

Urokinase Activity Fluorometric Assay Kit

Catalog Number **MAK185**
Storage Temperature –20 °C

TECHNICAL BULLETIN

Product Description

Urokinase (Urokinase-type plasminogen activator, uPA) is a serine protease that catalyzes the conversion of plasminogen to active plasmin. Urokinase is involved in diverse cellular processes including blood coagulation, cell adhesion, fibrinolysis, and cell migration. Studies in cultured fibroblasts have revealed urokinase induces MMP-9 expression and reactive oxygen species (ROS) generation.[1] Suppression of uPA expression has been studied for therapeutic implications in hepatocellular carcinoma (HCC).[2] Urokinase activity measurements may be useful for the study of cell functions and cancer therapy.

The Urokinase Activity Fluorometric Assay Kit provides a simple and direct procedure for measuring urokinase activity (ranging from 0.01–0.5 IU/well) in a variety of samples. Urokinase activity is determined using the enzymatic cleavage of an AMC-based (amido-methylcoumarin) peptide substrate, which results in the generation of AMC (λ_{ex} = 350/λ_{em} = 450 nm) proportional to the enzymatic activity present.

Components

The kit is sufficient for 100 assays in 96 well plates.

Urokinase Assay Buffer Catalog Number MAK185A	25 mL
Cell Lysis Buffer Catalog Number MAK185B	25 mL
Urokinase Substrate Catalog Number MAK185C	0.2 mL
Human Urokinase Catalog Number MAK185D	1 vl

Reagents and Equipment Required but Not Provided.

- 96 well flat-bottom plate – It is recommended to use black plates with clear bottoms for fluorescence assays.
- Fluorescence multiwell plate reader

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions

Briefly centrifuge vials before opening. To maintain reagent integrity, avoid repeated freeze/thaw cycles.

Urokinase Assay Buffer, Cell Lysis Buffer, and Urokinase Substrate – Allow buffers to come to room temperature before use.

Human Urokinase – Reconstitute with 1.1 mL of Urokinase Assay Buffer to generate 100 IU/mL Urokinase Standard Solution. Mix well by pipetting (do not vortex), then aliquot and store, protected from light at –80 °C. Use within 2 months of reconstitution.

Storage/Stability

The kit is shipped on wet ice and storage at –20 °C, protected from light, is recommended.

Procedure
All samples and standards should be run in duplicate.

Urokinase Standards for Fluorometric Detection

1. For High Urokinase Activity:
   Add 0, 1, 2, 3, 4, and 5 µL of Urokinase Standard Solution (100 IU/mL) into a 96 well plate, generating 0 (blank), 0.1, 0.2, 0.3, 0.4, and 0.5 IU/well standards. Add Urokinase Assay Buffer to each well to bring the volume to 50 µL.

2. For Low Urokinase Activity:
   Dilute 10 µL of the 100 IU/mL Urokinase Standard Solution with 90 µL of Urokinase Assay Buffer to prepare a 10 IU/mL (10 mIU/µL) Standard Solution. Add 0, 1, 2, 3, 4, and 5 µL of the 10 mIU/µL Standard Solution into a 96 well plate, generating 0 (blank), 10, 20, 30, 40, and 50 mIU/well standards. Add Urokinase Assay Buffer to each well to bring the volume to 50 µL.

Sample Preparation
Serum samples can be assayed directly.

Cells can be lysed in 4× cell volume of Cell Lysis Buffer. Incubate on ice for 5 minutes. Centrifuge the samples at maximum speed for 10 minutes at 4 °C. Collect the supernatant.

Note: For unknown samples, it is suggested to test several sample dilutions to ensure the readings are within the linear range of the standard curve. Dilute with Urokinase Assay Buffer if necessary.

Add 1–50 µL of sample per well. Bring samples to a final volume of 50 µL with Urokinase Assay Buffer.

Inherent fluorescence in samples can result in sample background. To correct for the background, include a Sample Blank for each sample by omitting the Urokinase Substrate. The Sample Blank readings can then be subtracted from the sample readings.

Assay Reaction

1. Set up Reaction Mixes according to the scheme in Table 1. 50 µL of Reaction Mix is required for each reaction (well).

Table 1.
Reaction Mixes

Reagent	Standards and Samples	Sample Blank
Urokinase Assay Buffer	48 µL	50 µL
Urokinase Substrate	2 µL	–

2. Add 50 µL of the appropriate Reaction Mix to each of well. Mix well using a horizontal shaker or by pipetting.

3. Incubate the plate at room temperature. After 2–3 minutes, take the initial measurement. Measure the fluorescence intensity ($FLU_{initial}$, λ_{ex} = 350/λ_{em} = 450 nm) at the initial time ($T_{initial}$).

4. Continue to incubate the plate at room temperature taking measurements (FLU) every 5 minutes. Protect the plate from light during the incubation.

5. The total incubation time will vary with the activity of the sample. Samples with low urokinase activity may need a longer incubation in order to detect enough fluorescence to register on the standard curve.

6. In order to obtain an accurate measurement, the ΔFLU (FLU_{final} – $FLU_{initial}$) must be in the linear range of the corrected fluorescence values used to create the standard curve. Continue to take readings until the ΔFLU for the sample falls on the standard curve.

Results

Calculations

Calculate the change in fluorescence measurement from $T_{initial}$ to T_{final} for all standards and samples to obtain the corrected fluorescence readings.

$$\Delta FLU = FLU_{final} - FLU_{initial}$$

Correct for background in the standards by subtracting the corrected fluorescence reading for the 0 (blank) Standard ($\Delta FLU_{Blank\ Standard}$) from each Standard. Use the values obtained from the appropriate Urokinase Standards to plot the standard curve.

Note: A new standard curve must be set up each time the assay is run.

Correct for background in the samples by subtracting the corrected fluorescence reading for the Sample Blank from each sample.

Note: All standards and samples must use the same $T_{initial}$ and T_{final}.

Using the corrected measurement, determine the Urokinase activity in the sample well from the standard curve.

Urokinase activity:

$$\text{Urokinase Activity (IU/mL)} = \frac{S_a}{S_v}$$

where:

S_a = Amount of Urokinase in unknown sample well from standard curve

S_v = sample volume (mL) added to well

Urokinase activity is reported as IU/mL.

Sample Calculation:

Urokinase Activity (S_a) = 0.384 (IU/well) (from standard curve)

Sample volume (S_v) = 0.05 mL

$$\frac{0.384\ \text{IU/well}}{0.050\ \text{mL/well}} = 7.7\ \text{IU/mL}$$

References

1. Zubkova, E.S. et al., Urokinase stimulates production of matrix metalloproteinase-9 in fibroblasts with involvement of reactive oxygen species. Bull. Exp. Biol. Med., **157(1)**, 18–21 (2014).
2. Yeh, C.B. et al., *Terminalia catappa* attenuates urokinase-type plasminogen activator expression through Erk pathways in Hepatocellular carcinoma. BMC Complement. Altern. Med., **14(1)**, 141 (2014).

Troubleshooting Guide

Problem	Possible Cause	Suggested Solution
Assay not working	Cold assay buffer	Assay Buffer must be at room temperature
	Omission of step in procedure	Refer and follow Technical Bulletin precisely
	Plate reader at incorrect wavelength	Check filter settings of instrument
	Type of 96 well plate used	For Fluorometric assays, use black plates with clear bottoms
Samples with erratic readings	Samples prepared in different buffer	Use the Assay Buffer provided or refer to Technical Bulletin for instructions
	Cell/Tissue culture samples were incompletely homogenized	Repeat the sample homogenization, increasing the length and extent of homogenization step.
	Samples used after multiple freeze-thaw cycles	Aliquot and freeze samples if needed to use multiple times
	Presence of interfering substance in the sample	If possible, dilute sample further
	Use of old or inappropriately stored samples	Use fresh samples and store correctly until use
Lower/higher readings in samples and standards	Improperly thawed components	Thaw all components completely and mix gently before use
	Use of expired kit or improperly stored reagents	Check the expiration date and store the components appropriately
	Allowing the reagents to sit for extended times on ice	Prepare fresh Reaction Mixes before each use
	Incorrect incubation times or temperatures	Refer to Technical Bulletin and verify correct incubation times and temperatures
	Incorrect volumes used	Use calibrated pipettes and aliquot correctly
Non-linear standard curve	Use of partially thawed components	Thaw and resuspend all components before preparing the Reaction Mixes
	Pipetting errors in preparation of standards	Avoid pipetting small volumes
	Pipetting errors in the Reaction Mix	Prepare Reaction Mixes whenever possible
	Air bubbles formed in well	Pipette gently against the wall of the plate well
	Standard stock is at incorrect concentration	Refer to the standard dilution instructions in the Technical Bulletin
	Calculation errors	Recheck calculations after referring to Technical Bulletin
	Substituting reagents from older kits/lots	Use fresh components from the same kit
Unanticipated results	Samples measured at incorrect wavelength	Check the equipment and filter settings
	Samples contain interfering substances	If possible, dilute sample further
	Sample readings above/below the linear range	Concentrate or dilute samples so readings are in the linear range

VI,KVG,LS,MAM 09/14-1