ab174098

Urokinase Activity Assay Kit (Fluorometric)

Instructions for Use

For the sensitive and accurate measurement of Urokinase activity in a variety of samples

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

Version: 1 Last Updated: 05 August 2013

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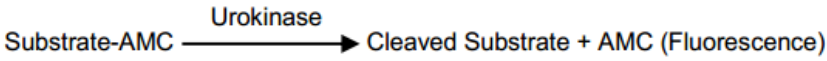
1. Overview

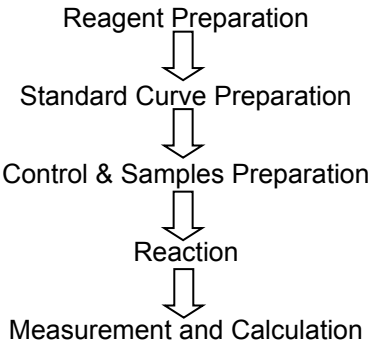
Urokinase (urokinase-type plasminogen activator or uPA, EC 3.4.21.73) is a serine protease, which catalyzes the conversion of the plasminogen to active plasmin, which in turn participates in thrombolysis.

Abcam's Urokinase Fluorimetric Activity Assay Kit (ab174098) utilizes an AMC-based peptide substrate containing the recognition sequence for Urokinase. Urokinase present in the sample catalyzes the cleavage of the substrate and releases AMC, which can be easily quantified by measuring its fluorescence at Ex/Em = 350/450 nm. This sensitive assay detects very low levels of Urokinase activity in biological samples (as low as 0.02 IU/mL).

Figure 1: Assay Procedure.



2. Protocol Summary



3. Kits Components

Item	Quantity
Urokinase Assay Buffer	25 mL
Cell Lysis Buffer	25 mL
Urokinase Substrate	0.2 mL
Human Urokinase (Standard/Positive Control)	1 vial

4. Storage and Stability

Upon arrival, store kit at –20°C and protect from light.

Briefly centrifuge small vials at low speed prior to opening. Read the entire protocol before performing the experiment.

5. Materials Required, Not Supplied

- 96-well black plate with flat bottom (for fluorometric reading)
- Multi-well spectrophotometer (ELISA reader)
- Multi-channel pipette
- Distilled water

6. Reagents Preparation

1. **Urokinase Assay Buffer and Cell Lysis Buffer:**
   Ready to use as supplied. Warm to room temperature before use. Store at -20°C.

2. **Urokinase Substrate:**
   Ready to use as supplied. Warm to room temperature before use. Store at -20°C.

3. **Human Urokinase (Standard/Positive Control):**
   Reconstitute with 1.1 mL of Urokinase Assay Buffer to prepare Urokinase stock solution of 100 IU/mL. Mix well, do not vortex. Aliquot and store at -80°C. Avoid repeated freeze/thaw. Use within two months.

7. Assay Protocol

1. **Sample Preparation**

a) **Serum:**
   Serum samples can be diluted with Urokinase Assay Buffer and measured directly.
   Add 1 – 50 µL test sample to wells of a 96-well plate. If volume needed is < 50 µL, bring it up to 50 µL with Assay Buffer.

b) Cellular Extracts:

Collect 10^6 – 2x 10^6 adherents or suspension cells (by trypsinization or centrifugation) and discard supernatant. Rapidly lyse cells by resuspending them with 4X cell volume of ice cold Cell Lysis Buffer and pipetting up and down using a smaller tip. Incubate on ice for 5 minutes. Centrifuge at maximum speed (12000rpm) for 10 minutes at 4°C. Collect the supernatant.

Add 1-50 µL test sample to wells of a 96-well plate. If volume needed is <50 µL, bring it up to 50 µL with Cell Lysis Buffer.

c) Nuclear Extracts:

For preparing nuclear extracts, we recommend using EpiSeeker Nuclear Extraction Kit (ab113474) or a similar method.

NOTE: For unknown samples, we recommend making several dilutions of your samples to ensure that the values for unknowns fall within the limits of the Standard Curve.

d) Purified enzyme:

The concentration of the purified Urokinase used per assay will depend on the activity of the purified enzyme.

Prepare a series of dilutions of the purified enzyme to be able to compare activity with the Human Urokinase Positive Control provided with the kit.

Add 50 µL of purified diluted protein to wells of a 96-well plate.

e) BACKGROUND CONTROL:

For samples having high fluorescence background, prepare parallel sample well(s) as sample background control.

2. Standard Curve

The set up of the standard curve will depend on whether the samples are expected to have high or low activity. Consult literature about your sample type, or set up both type of standard curves to ensure you cover all possibilities.

a) For high urokinase activity:

Using the 100 IU/mL Urokinase Stock Standard solution, prepare standard curve dilution as follows, in a microplate or microcentrifuge tubes:

Urokinase 100 IU/mL amount (µl)	Urokinase assay buffer (µL)	Amount in well	UROKINASE ACTIVITY IN WELL
0	150	50 µl	0 IU/well
3	1447	50 µl	0.1 IU/well
6	144	50 µl	0.2 IU/well
9	141	50 µl	0.3 IU/well
12	138	50 µl	0.4 IU/well
15	135	50 µl	0.5 IU/well

Add 50 µL of each standard dilution into a well in a 96-well plate to set up standard. Each dilution had enough amount of standard to set up 2 duplicates x 50 µL/well.

b) For low urokinase activity:

Dilute the 100 IU/mL Urokinase Standard to 10 IU/mL by adding 10 µL of 100 IU/mL Urokinase Standard solution to 90 µL of Urokinase Assay Buffer.

Urokinase 10 IU/mL amount (µl)	**Urokinase assay buffer (µL)**	**Amount in well**	**UROKINASE ACTIVITY IN WELL**
0	150	50 µl	0 IU/well
3	1447	50 µl	0.01 IU/well
6	144	50 µl	0.02 IU/well
9	141	50 µl	0.03 IU/well
12	138	50 µl	0.04 IU/well
15	135	50 µl	0.05 IU/well

Add 50 µL of each standard dilution into a well in a 96-well plate to set up standard. Each dilution had enough amount of standard to set up 2 duplicates x 50 µL/well.

3. Reaction Mix:

Prepare Reaction Mix for each reaction:

	Reaction Mix
Urokinase Assay Buffer	48 µL
Urokinase Substrate	2 µL

Mix enough reagents for the number of assays (samples, standards and background control) to be performed. Prepare a Master Mix of the Reaction Mix to ensure consistency. We recommend the following calculation:

	Reaction Mix
Urokinase Assay Buffer	48 µL x (Nb samples + Standards + 1)
Urokinase Substrate	2 µL x (Nb samples + Standards + 1)

4. Plate set up and Detection:

a) Add 50 µL of standard and sample to wells.

b) Add 50 µL of Reaction Mix to each well containing the Standard and test sample wells. Mix well.

c) Optional: If you suspect your samples will give high fluorescence, add 100 µL of Background Control samples to wells.

d) Immediately measure fluorescence (**RFU_1**) at Ex/Em = 350/450 nm using a fluorescence microplate reader. Incubate for 30-60 min at room temperature and measure fluorescence again (**RFU_2**).

NOTE: Incubation time depends on the Urokinase activity in the samples. Longer incubation times may be required to generate sufficient fluorescence signal for samples having very low activity.

8. Data Analysis

Calculations:

a) Subtract the reading at time zero (Δ**RFU = RFU$_2$ – RFU$_1$**) from all Samples (ΔRFU_S) and Urokinase Standards (ΔRFU_{UK}).

b) Obtain the corrected fluorescence readings for Urokinase Standards by subtracting the zero Standard reading from all Standards (ΔRFU_{UK}- $\Delta RFU_{0Standard}$).

Note: If the sample background control reading is significant, subtract the sample background control fluorescence from that of corrected sample reading.

c) Plot the Urokinase activity Standard Curve. Calculate the corrected fluorescence for all the test samples (**ΔRFU_S –** Δ**RFU$_{0Standard}$**).

d) Using the corrected fluorescence reading of test samples and the equation obtained from Urokinase Standards Curve, calculate the activity (IU/mL) of the test samples.

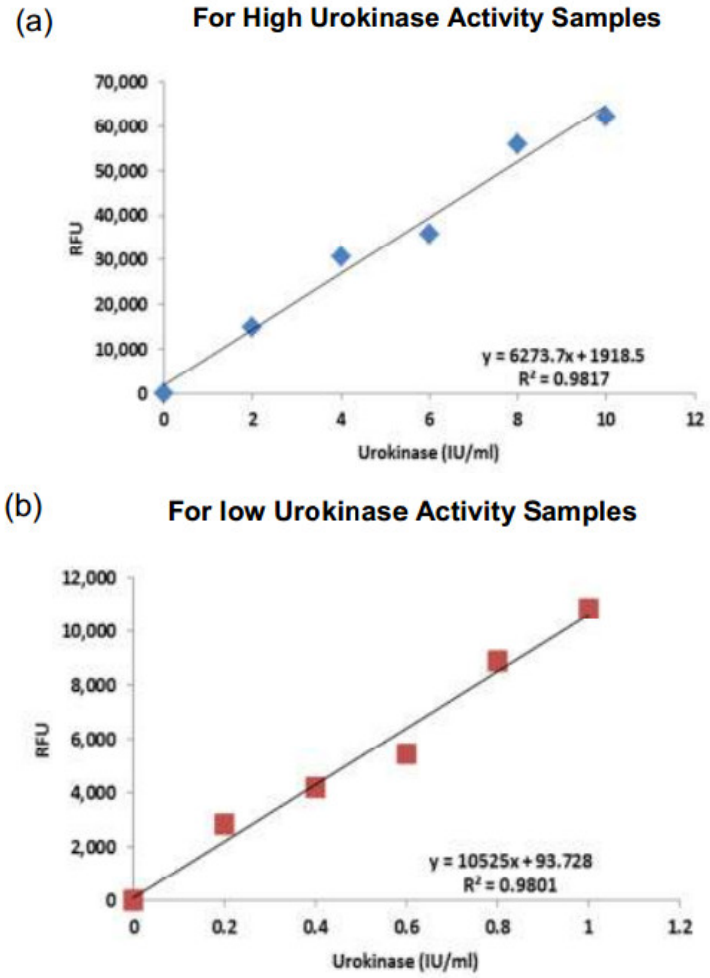


Figure 2: Urokinase Activity Standard Curves, **(a)** for high Urokinase activity samples and **(b)** for low Urokinase activity samples. Assays were performed following the kit protocol.

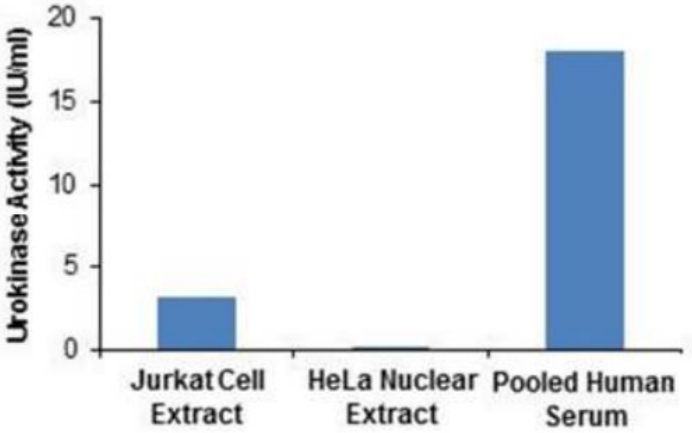


Figure 3: Urokinase activities of Jurkat cell extract (10 µL), Hela nuclear extract (10 µL) and pooled human serum (10 µL). Assays were performed following the kit protocol.

9. Troubleshooting

Problem	Reason	Solution
Assay not working	Assay buffer at wrong temperature	Assay buffer must not be chilled - needs to be at RT
	Protocol step missed	Re-read and follow the protocol exactly
	Plate read at incorrect wavelength	Ensure you are using appropriate reader and filter settings (refer to datasheet)
	Unsuitable microtiter plate for assay	Fluorescence: Black plates (clear bottoms); Luminescence: White plates; Colorimetry: Clear plates. If critical, datasheet will indicate whether to use flat- or U-shaped wells
Unexpected results	Measured at wrong wavelength	Use appropriate reader and filter settings described in datasheet
	Samples contain impeding substances	Troubleshoot and also consider deproteinizing samples
	Unsuitable sample type	Use recommended samples types as listed on the datasheet
	Sample readings are outside linear range	Concentrate/ dilute samples to be in linear range

Problem	Reason	Solution
Samples with inconsistent readings	Unsuitable sample type	Refer to datasheet for details about incompatible samples
	Samples prepared in the wrong buffer	Use the assay buffer provided (or refer to datasheet for instructions)
	Samples not deproteinized (if indicated on datasheet)	Use the ***10kDa spin column (ab93349)***
	Cell/ tissue samples not sufficiently homogenized	Increase sonication time/ number of strokes with the Dounce homogenizer
	Too many freeze-thaw cycles	Aliquot samples to reduce the number of freeze-thaw cycles
	Samples contain impeding substances	Troubleshoot and also consider deproteinizing samples
	Samples are too old or incorrectly stored	Use freshly made samples and store at recommended temperature until use
Lower/ Higher readings in samples and standards	Not fully thawed kit components	Wait for components to thaw completely and gently mix prior use
	Out-of-date kit or incorrectly stored reagents	Always check expiry date and store kit components as recommended on the datasheet
	Reagents sitting for extended periods on ice	Try to prepare a fresh reaction mix prior to each use
	Incorrect incubation time/ temperature	Refer to datasheet for recommended incubation time and/ or temperature
	Incorrect amounts used	Check pipette is calibrated correctly (always use smallest volume pipette that can pipette entire volume)

Problem	Reason	Solution
Standard curve is not linear	Not fully thawed kit components	Wait for components to thaw completely and gently mix prior use
	Pipetting errors when setting up the standard curve	Try not to pipette too small volumes
	Incorrect pipetting when preparing the reaction mix	Always prepare a master mix
	Air bubbles in wells	Air bubbles will interfere with readings; try to avoid producing air bubbles and always remove bubbles prior to reading plates
	Concentration of standard stock incorrect	Recheck datasheet for recommended concentrations of standard stocks
	Errors in standard curve calculations	Refer to datasheet and re-check the calculations
	Use of other reagents than those provided with the kit	Use fresh components from the same kit

For further technical questions please do not hesitate to contact us by email (technical@abcam.com) or phone (select "*contact us*" on www.abcam.com for the phone number for your region).

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