

ab326739 – Mouse uPA SimpleStep ELISA® Kit – Chemiluminescent

For the quantitative measurement of uPA in mouse serum, plasma (heparin), plasma (EDTA), plasma (citrate), urine, and cell culture media.

For research use only - not intended for diagnostic use.

Patent pending.

For overview, typical data and additional information please visit: www.abcam.com/ab326739

Storage and Stability: Store kit at 2-8°C immediately upon receipt. Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in the Standard Preparation and Reagent preparation sections.

Limitations: All data, except typical standard curve and sensitivity were collected using the colorimetric version of this kit (ab245727).

Materials Supplied

Item	Quantity 1 x 96 tests	Storage Condition
Mouse uPA Capture Antibody 10X	600 µL	+4°C
Mouse uPA Detector Antibody 10X	600 µL	+4°C
Mouse uPA Lyophilized Recombinant Protein	2 Vials	+4°C
Antibody Diluent 4BR	6 mL	+4°C
Sample Diluent NS	50 mL	+4°C
Wash Buffer PT 10X	20 mL	+4°C
ChemiHRP Reagent A	3 mL	+4°C
ChemiHRP Reagent B	3 mL	+4°C
SimpleStep Pre-Coated Black 96-Well Microplate	96 Wells	+4°C
Plate Seal	1	+4°C

Materials Required, Not Supplied

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

Luminometer with the following settings: 0.5-1 second/well read time; summation mode (all wavelengths).

Deionized water.

Multi- and single-channel pipettes.

Tubes for standard dilution.

Orbital microplate shaker for all incubation steps: capable of 750 rpm shaking speed.

Optional: Phenylmethylsulfonyl Fluoride (PMSF) (or other protease inhibitors).

Reagent Preparation

Equilibrate all reagents to room temperature (18-25°C) prior to use. The kit contains enough reagents for 96 wells. The sample volumes below are sufficient for 48 wells (6 x 8-well strips); adjust volumes as needed for the number of strips in your experiment.

Prepare only as much reagent as is needed on the day of the experiment. Capture and Detector Antibodies have only been tested for stability in the provided 10X formulations.

1X Wash Buffer PT: Prepare 1X Wash Buffer PT by diluting Wash Buffer PT 10X with deionized water. To make 50 mL 1X Wash Buffer PT combine 5 mL Wash Buffer PT 10X with 45 mL deionized water. Mix thoroughly and gently.

Antibody Cocktail: Prepare Antibody Cocktail by diluting the capture and detector antibodies in Antibody Diluent 4BR. To make 3 mL of the Antibody Cocktail combine 300 µL 10X Capture Antibody and 300 µL 10X Detector Antibody with 2.4 mL Antibody Diluent 4BR. Mix thoroughly and gently.

Lumi HRP Development Solution: Just prior to use, prepare Lumi HRP Development Solution by mixing equal volume of the ChemiHRP Reagent A and the ChemiHRP Reagent B. To make 3 mL of the Lumi HRP Development Solution combine 1.5 mL of ChemiHRP Reagent A and 1.5 mL of ChemiHRP Reagent B. Mix thoroughly and gently by inversion or slow pipetting (Avoid shaking or vortexing). Protect the prepared solution from light until use.

Standard Preparation

Always prepare a fresh set of standards for every use. Discard working standard dilutions after use as they do not store well. The following section describes the preparation of a standard curve for duplicate measurements (recommended).

- Reconstitute the uPA standard sample by adding the volume of Sample Diluent NS indicated on the protein vial label. Hold at room temperature for 10 minutes. Mix thoroughly and gently. This is the 37,100 pg/mL **Stock Standard** Solution.
- Label nine tubes, Standards 1– 9.
- Add 187 µL of Sample Diluent NS into tube number 1 and 150 µL of Sample Diluent NS into numbers 2-9.
- Use the **Stock Standard** to prepare the following dilution series. Standard #9 contains no protein and is the Blank control:

Standard #	Dilution Sample	Volume to Dilute (µL)	Volume of Diluent (µL)	Starting Conc. (pg/mL)	Final Conc. (pg/mL)
1	Stock Standard	69	187	37,100	10,000
2	Standard#1	75	150	10,000	3,333.3
3	Standard#2	75	150	3,333.3	1,111.1
4	Standard#3	75	150	1,111.1	370.4
5	Standard#4	75	150	370.4	123.5
6	Standard#5	75	150	123.5	41.2
7	Standard#6	75	150	41.2	13.7
8	Standard#7	75	150	13.7	4.6
9	Blank Control	0	150	0	0

Sample Preparation

Typical Sample Dynamic Range	
Sample Type	Range
Serum	3.13% - 50%
Plasma-Citrate	3.13% - 50%
Plasma-EDTA	1.56% - 25%
Plasma-Heparin	1.56% - 50%
Urine	0.094% - 1.5%
Cell culture media*	≤ 50%

*Based on spiked sample

Serum Samples should be collected into a serum separator tube. After clot formation, centrifuge samples at 2,000 x g for 10 minutes and collect serum. Dilute samples at least 1:2 into Sample Diluent NS and assay. Store un-diluted serum at -20°C or below. Avoid repeated freeze-thaw cycles.

Plasma Collect plasma using citrate, EDTA or heparin. Centrifuge samples at 2,000 x g for 10 minutes. Dilute Plasma-Citrate and Plasma-Heparin samples at least 1:2 and Plasma-EDTA samples at least 1:4 into Sample Diluent NS and assay. Store un-diluted plasma samples at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.

Cell Culture Supernatants Centrifuge cell culture media at 2,000 x g for 10 minutes to remove debris. Collect supernatants and dilute samples at least 1:2 into Sample Diluent NS and assay. Store un-diluted samples at -20°C or below. Avoid repeated freezethaw cycles.

Urine Centrifuge urine at 2,000 x g for 10 minutes to remove debris. Dilute samples at least 1:66.67 into Sample Diluent NS and assay. Store un-diluted urine samples at -20°C or below. Avoid repeated freeze-thaw cycles.

Plate Preparation

The 96 well plate strips included with this kit are supplied ready to use. It is not necessary to rinse the plate prior to adding reagents.

Unused plate strips should be immediately returned to the foil pouch containing the desiccant pack, resealed and stored at 4°C.

For each assay performed, a minimum of two wells must be used as the zero control.

For statistical reasons, we recommend each sample should be assayed with a minimum of two replicates (duplicates).

Differences in well absorbance or "edge effects" have not been observed with this assay.

Assay Procedure

Equilibrate all materials and prepared reagents to room temperature prior to use.

We recommend that you assay all standards, controls and samples in duplicate.

1. Prepare all reagents, working standards, and samples as directed in the previous sections.
2. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, reseal and return to 4°C storage.

3. Add 50 µL of all sample or standard to appropriate wells.
4. Add 50 µL of the Antibody Cocktail to each well.
5. Seal the plate and incubate for 30 minutes at room temperature on a plate shaker set to 750 rpm.
6. Wash each well with 3 x 350 µL 1X Wash Buffer PT. Wash by aspirating or decanting from wells then dispensing 350 µL 1X Wash Buffer PT into each well. Wash Buffer PT should remain in wells for at least 30 seconds. Complete removal of liquid at each step is essential for good performance. After the last wash invert the plate and tap gently against clean paper towels to remove excess liquid.
7. Add 50 µL of prepared Lumi HRP Development Solution to each well and incubate for 1 minute in the dark on a plate shaker set to 750 rpm. Further optimization of incubation time vs signal strength can be performed if needed. Avoid introducing bubbles into the wells.
8. Measure the produced light of each well using a microplate luminometer with the following settings: 0.5-1 second/well read time in summation mode (all wavelengths). Relative light unit (RLU) readings may vary between luminometer models. It is recommended to configure instrument settings according to the manufacturer's specifications. Note: Relative light unit (RLU) values may change over the course of the 15-minute reading window.
9. Analyze the data as described below.

Mode:	Luminescence
Instrument settings:	Endpoint
Detection Mode:	All wavelengths
Read Time:	0.5-1 sec
Read:	Top

Note For microplate readers with Pre-Read Optimization option, the Read Height as well as Microplate Optimization is recommended before the first read.

Download our ELISA guide for technical hints, results, calculation, and troubleshooting tips:

<https://www.abcam.com/en-us/technical-resources/guides/elisa-guide>

Technical Support

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Version 1a | 2026-05-08

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Additional information

ASSAY SPECIFICITY

This kit is designed for the quantification of mouse uPA.

The standard protein in this kit is an N-terminal fragment of mouse uPA.

Native signal was detected in serum, plasma (citrate), plasma (EDTA), plasma (heparin), and urine sample types.

Spiked protein experiments were used to validate cell culture supernatant sample types.

Saliva, milk, CSF, cell extract, and tissue extract samples have not been tested with this kit.

CROSS REACTIVITY

Recombinant Mouse tPA, PAI, and Plasminogen were prepared at 50 ng/mL and assayed for cross reactivity. No cross-reactivity was observed.

INTERFERENCE

Recombinant Mouse tPA, PAI, and Plasminogen were prepared at 50 ng/mL and tested for interference. No interference with was observed.

SPECIES REACTIVITY

Other species reactivity was determined by measuring 25% serum samples of various species, interpolating the protein concentrations from the mouse standard curve, and expressing the interpolated concentrations as a percentage of the protein concentration in mouse serum assayed at the same dilution.

Reactivity was determined for the following species: Rat (12%).
Reactivity <3% was determined for the following species: Human, Cow.
Other species reactivity not determined.

CALCULATION

- Preconfigured protocols are available when using SoftMax Pro software from Molecular Devices.
- Calculate the average chemiluminescence value for the blank control (zero) standards. Subtract the average blank control standard chemiluminescence value from all other chemiluminescence values.
- Create a standard curve by plotting the average blank control subtracted chemiluminescence value for each standard concentration (y-axis) against the target protein concentration (x-axis) of the standard. Use graphing software to draw the best smooth curve through these points to construct the standard curve.
- Note: Most chemiluminescence reader software or graphing software will plot these values and fit a curve to the data. A four-parameter curve fit (4PL) is often the best choice; however, other algorithms (e.g. linear, semi-log, log/log, 4-parameter logistic) can also be tested to determine if it provides a better curve fit to the standard values.
- Determine the concentration of the target protein in the sample by interpolating the blank control subtracted chemiluminescence values against the standard curve. Multiply the

resulting value by the appropriate sample dilution factor, if used, to obtain the concentration of target protein in the sample.

- Samples generating chemiluminescence values greater than that of the highest standard should be further diluted and reanalyzed. Similarly, samples which measure at chemiluminescence values less than that of the lowest standard should be retested in a less dilute form.

TYPICAL DATA

Typical standard curve – data provided for demonstration purposes only. A new standard curve must be generated for each assay performed.

Standard Curve Measurements			
Concentration (pg/mL)	RLU		Mean RLU
	1	2	
0	844	850	847
4.6	2,204	1,855	2,030
13.7	4,513	4,514	4,514
41.2	14,306	14,893	14,600
123.5	38,642	41,445	40,044
370.4	144,480	157,770	151,125
1,111.1	474,860	463,640	469,250
3,333.3	1,678,100	1,607,600	1,642,850
10,000	5,132,600	5,229,000	5,180,800

Table 1. Example of mouse uPA standard curve in Sample Diluent NS. The uPA standard curve was prepared as described in the Standard Preparation section. The table shows raw data values.

TYPICAL SAMPLE VALUES

Sensitivity:

The calculated minimal detectable dose (MDD) is 1.9 pg/mL. The MDD was determined by calculating the mean of zero standard replicates (n=16) and adding 2 standard deviations then extrapolating the corresponding concentration.

Recovery

Three concentrations of uPA were spiked in duplicate to the indicated biological matrix to evaluate signal recovery in the working range of the assay.

Sample Type	Average % Recovery	Range (%)
25% Serum	109	103-118
25% Plasma-Citrate	106	104-106
25% Plasma-EDTA	113	108-120
12.5% Plasma-Heparin	110	105-114
1:400 Urine	112	101-124
50% Cell culture media*	95	91-99

*Media is DMEM containing 10% fetal bovine serum.

Linearity of Dilution

Linearity of dilution is determined based on interpolated values from the standard curve. Linearity of dilution defines a sample concentration interval in which interpolated target concentrations are directly proportional to sample dilution.

Native uPA was measured in the following biological samples in a 2-fold dilution series. Sample dilutions are made in Sample Diluent NS.

Dilution Factor	Interpolated value	50% Mouse Serum	50% Mouse Plasma (Citrate)	50% Mouse Plasma (EDTA)	50% Mouse Plasma (Heparin)	1.5% Mouse Urine
Undiluted	pg/mL	3,487	2,840	1,775	2,561	4,011
	% Expected value	100	100	100	100	100
2	pg/mL	1,891	1,527	766	1,233	2,038
	% Expected value	108	108	86	96	102
4	pg/mL	908	768	529	649	956
	% Expected value	104	108	119	101	95
8	pg/mL	438	410	205	283	522
	% Expected value	100	115	92	89	104
16	pg/mL	220	207	110	149	272
	% Expected value	101	117	99	93	109

Recombinant uPA was spiked into the following biological samples and then diluted in a 2-fold dilution series. Sample dilutions are made in Sample Diluent NS.

Dilution Factor	Interpolated value	50% Cell Culture Media
Undiluted	pg/mL	926.2
	% Expected value	100
2	pg/mL	452.8
	% Expected value	98
4	pg/mL	232.9
	% Expected value	101
8	pg/mL	107.1
	% Expected value	93
16	pg/mL	49.7
	% Expected value	86

Precision

Mean coefficient of variations of interpolated values of uPA from a single concentration of mouse serum within the working range of the assay.

	Intra-assay	Inter-assay
N=	8	3
CV (%)	2.8	3.5

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