ab83355 – ATP Assay Kit (Colorimetric/Fluorometric)

For the rapid, sensitive and accurate measurement of ATP in a variety of samples.
For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit: www.abcam.com/ab83355
(use www.abcam.cn/ab83355 for China, or www.abcam.co.jp/ab83355 for Japan)

Storage and Stability: Store kit at -20°C in the dark on receipt. Kit can be stored for 1 year if components have not been reconstituted. Reconstituted components are stable for 2 months. Aliquot components in working volumes before storing at the recommended temperature. Avoid repeated freeze-thaws of reagents.

Materials Supplied:

Item	Quantity	Storage temperature (before prep)	Storage temperature (after prep)
ATP Assay Buffer	25 mL	-20°C	-20°C or 4°C
ATP Probe	200 µL	-20°C	-20°C. Protect from light.
ATP Converter	1 vial	-20°C	-20°C
Developer Mix	1 vial	-20°C	-20°C
ATP Standard	1 vial	-20°C	-20°C.

Materials Required, Not Supplied

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

- Microplate reader capable of measuring absorbance at OD 570 nm (colorimetric) or fluorescence at Ex/Em = 535/587 nm (fluorometric)
- 96 well plate with clear flat bottom (for colorimetric assay) / 96 well plate with clear flat bottom, preferably black (for fluorometric assay)
- Dounce homogenizer (if using tissue)
- Deproteinizing Sample Preparation Kit – TCA (ab204708): for deproteinization step in cell or tissue samples
- 10 kD Spin columns (ab93349): for deproteinization step in fluid samples
- (Optional) Red Blood Cell (RBC) Lysis Buffer (ab204733): for lysis of red blood cells.

Optional – for highly metabolic tissues:

- Perchloric acid (2N), Ice cold
- Potassium hydroxide (KOH), 2M.

For Alternative deproteinization protocol

- Perchloric acid (PCA) 4M, ice cold
- Potassium hydroxide (KOH), 2M

Reagent Preparation:

- Briefly centrifuge small vials at low speed prior to opening.

ATP Assay Buffer: Ready to use as supplied. Equilibrate to Room temperature (RT).
ATP Standard (lyophilized, 1 µmol): Reconstitute in 100 µL of ddH_2O to generate a 10 mM ATP standard stock solution. Keep on ice while in use.
ATP Probe (in DMSO): Warm by placing in a 37°C bath for 1 – 5 min to thaw the DMSO solution before use. Repeat this step every time probe is needed.
ATP Converter and **Developer Mix (lyophilized):** Dissolve in 220 µL ATP Assay Buffer. Keep on ice during the assay.

Standard Preparation

- Always prepare a fresh set of standards for every use.
- Discard working standard dilutions after use as they do not store well.

For Colormetric Assay

Prepare 100 µL of 1 mM ATP standard by diluting 10 µL of the provided ATP Standard (10 mM solution) with 90 µL of ddH2O.

For fluorometric assay

Prepare a 1 mM ATP standard by diluting 5 µL of the provided ATP Standard (10 mM solution) with 45 µL of ddH2O. Prepare 100 µL of 0.1 mM ATP standard by diluting 10 µL of 1 mM ATP standard with 90 µL of ddH2O.

Δ Note: Detection sensitivity of fluorometric assay is 10-fold higher than colorimetric assay Using the appropriate ATP working standard prepare standard curve dilution as described in the table. Each dilution has enough standard to set up duplicate readings (2 x 50 µL).

Standard #	ATP 1mM Standard (µL)	Assay Buffer (µL)	Final volume standard in well (µL)	End amount ATP (nmol/well)	
				Colorimetric	Fluorometric
1	0	150	50	0	0
2	6	144	50	2	0.2
3	12	138	50	4	0.4
4	18	132	50	6	0.6
5	24	126	50	8	0.8
6	30	120	50	10	1.0

Sample Preparation

- Perform several dilutions of sample to ensure readings are within standard value range.
- Use fresh samples or snap freeze samples in liquid nitrogen upon extraction and store immediately at -80°C. When you are ready to test samples, thaw on ice. Note: this might affect the stability of samples, and readings can be lower than expected
- Endogenous compounds in the sample may interfere with the reaction. To ensure accurate determination of ATP in the test samples, we recommend spiking samples with a known amount of Standard (initial recommendation: 300 pmol).
- The kit may be used with living cells, Saccharomyces cerevisiae (yeast) or cell medium, the optimal standardized conditions however must be empirically determined.

Notes: Cell and tissue samples may contain enzymes which interfere with the assay. You can remove these enzymes from cell samples by using Deproteinizing Sample Preparation Kit – TCA (ab204708). For both tissue and cell samples you can perform a PCA/KOH deproteinization step following the protocol described later on.

Cells lysates:

1. Harvest cells for each assay (initial recommendation: 1x106 cells) & wash with cold PBS.
2. Resuspend cells in 100 µL of ATP Assay Buffer.
3. Homogenize cells quickly by pipetting up and down a few times.
4. Centrifuge 5 minutes at 4°C at 13,000 g in a cold microcentrifuge to remove any insoluble material.
5. Collect supernatant and transfer to a new tube and keep on ice.
6. Remove enzymes if present from sample that may interfere with assay by using Deproteinizing Sample Preparation Kit – TCA (ab204708).

Tissue lysates:

Note: For highly metabolically active tissues such as muscle, we recommend preparing tissue samples directly in PCA following the alternative protocol. If you don't have access to PCA, then proceed with the instructions described below.

7. Harvest tissue necessary for each assay (initial recommendation: 10 mg) & wash in cold PBS.
8. Homogenize tissue in 100 µL of ATP Assay Buffer with a Dounce homogenizer (preferred for better assay reproducibility) or pestle sitting on ice, with 10-15 passes.
9. Centrifuge sample for 2-5 minutes at 4°C at 13,000 g using a cold microcentrifuge to remove any insoluble material.
10. Collect supernatant and transfer to a new tube and keep on ice.

Highly metabolically active tissues – alternative protocol:

Note: Glycerol phosphate is a natural by-product of glycolysis, especially abundant in highly metabolic tissues, and it is very difficult to remove from samples. Dilution of the sample in assay buffer will dilute the effect of the glycerol phosphate. Setting up a background control sample will allow to discard any effect caused by the glycerol phosphate.

1. Harvest the amount of tissue necessary for each assay (initial recommendation: 10 mg tissue) and wash tissue in cold PBS.
2. Homogenize tissue in100 µL ice cold 2N PCA with a Dounce homogenizer sitting on ice, with 10-15 passes. Keep samples on ice for 30-45 minutes.
3. Centrifuge samples at 13,000 g for 2 minutes at 4°C in a cold centrifuge and transfer supernatant to a fresh tube. Measure volume of supernatant.
4. After homogenizing the tissue in 100 µL of PCA, dilute the volume to 500 µL with the ATP Assay Buffer. ab83355 ATP Assay Kit
5. Precipitate excess PCA by adding ice-cold 2M KOH that equals 10-20% of the total volume and vortexing briefly. This will neutralize the sample and precipitate excess PCA.
6. It is important to test pH during neutralization: pH should equal 6.5-8 (use pH paper to test 5 µL of sample). If necessary, adjust pH with 0.1 M KOH or PCA.
7. Centrifuge at 13,000 g for 15 minutes at 4°C and collect supernatant.
8. Samples are now deproteinized, neutralized and PCA has been removed. The samples are now ready to use in the assay. No additional process is required.
9. To calculate the dilution factor introduced by the deproteinization step (DDF), simply apply the following formula:

$$DDF = \frac{500\ \mu L\ + volume\ KOH\ (\mu L)}{initial\ sample\ volume\ in\ PCA}$$

Plasma, Serum and Urine (and other biological fluids):

Use heparin when collecting plasma or serum. EDTA and other chelators should be avoided. protein which can interfere with the assay can be deproteinized with our 10 kD Spin Columns (ab93349) to deproteinize biological fluids.

1. Add sample to the spin column, centrifuge at 13,000 xg for 10 min at 4°C. Collect the filtrate.
2. The find the optimal values and ensure your readings will fall within the standard values, we recommend performing several dilutions of the sample (initial recommendations: 1:2 – 1:5 – 1:10).

Red blood cells (RBC):

We recommend harvesting 1 x 10^7 RBCs as they are generally smaller cells.

RBC sample can be prepared using Red Blood Cell (RBC) Lysis Buffer (ab204733) following by a deproteinization step using Deproteinizing Sample Preparation Kit – TCA (ab204708).

If not using RBC Lysis Buffer, red blood cells can be lysed using the following protocol:

1. Homogenize sample in 100 µL of ATP Assay Buffer.
2. Lyse cells by snap freeze-thaw cycles.
3. Centrifuge sample for 2 minutes at 4°C at 13,000 xg using a microcentrifuge to remove any insoluble material.
4. Collect supernatant and transfer to a clean tube.
5. Keep on ice.
6. Remove interfering enzymes from sample by using Deproteinizing Sample Preparation Kit – TCA (ab204708).
7. Alternatively, you can perform a PCA/KOH deproteinization step following the protocol described below.

Red blood cells preserved in glycerol are suitable for the assay as long as deglycerolization is performed before running the assay.

Alternative deproteinization protocol:

You should have a clear protein sample after homogenization and centrifugation. Keep your samples on ice.

1. Add ice cold PCA 4 M to a final concentration of 1 M in the homogenate solution and vortex briefly to mix well.

Δ **Note:** high protein concentration samples might need more PCA.

2. Incubate on ice for 5 minutes.
3. Centrifuge samples at 13,000 xg for 2 minutes at 4°C in a cold centrifuge and transfer supernatant to a fresh tube. Measure volume of supernatant.
4. Precipitate excess PCA by adding ice-cold 2M KOH that equals 20-35% of the total volume (sample +PCA) and vortexing briefly. This will neutralize the sample and precipitate excess PCA.
5. After neutralization, it is very important that pH equals 6.5-8 (use pH paper to test 1 µL of sample). If necessary, adjust pH further with 0.1 M KOH or PCA.
6. Centrifuge at 13,000 xg for 15 minutes at 4°C and collect supernatant.
7. Samples are now deproteinized, neutralized and PCA has been removed. The samples are now ready to use in the assay.
8. To calculate the dilution factor introduced by the deproteinization step (DDF), simply apply the following formula:

$$DDF = \frac{initial\ sample\ volume\ + volume\ PCA + volume\ KOH\ (\mu L)}{initial\ sample\ volume\ in\ PCA}$$

Δ **Note:** We suggest using different volumes of sample to ensure readings are within the standard curve range.

Δ **Note:** The PCA extracts should be neutralized as soon as possible, as the acid conditions, also cause hydrolysis of the cyclic phosphates and may isomerize other phosphates.

Assay Procedure – Colorimetric & Fluorometric Assay

- Equilibrate all materials and prepared reagents to room temperature just prior to use and gently agitate.
- Assay all standards, controls and samples in duplicate.

For fluorometric assays, use probe 1:10 diluted reduces fluorescence background.

Δ **Note**: Glycerol phosphate present in cell or tissue extracts can generate background in this assay. If you suspect your samples contain glycerol phosphate, set up Sample Background Controls.

Plate Loading (clear plate):

For colorimetric use clear plates and for Fluorometric use black walled, clear bottom plates.

- Standard wells = 50 µL standard dilutions.
- Sample wells = 1-50 µL samples (adjust volume to 50 µL/well with ATP Assay Buffer).
- Sample Background Control wells = 1-50 µL samples (adjust volume to 50µL/well with ATP Assay Buffer).

The need to run background controls for every sample depends on sample type and background signal. Ie for diverse samples with high background, run controls for all samples. For uniform samples, it may be possible to run controls for only one representative sample. Run background control wells, each time you run the assay.

ATP reaction mix:

1. Prepare 50 µL of ATP Reaction Mix and Background Control Mix for each reaction. Mix enough reagents for the number of assays to be performed. Prepare a master mix to ensure consistency.

Component	Reaction Mix (µL)		Background Reaction Mix (µL)	
	Colorimetric	Fluorometric	Colorimetric	Fluorometric
ATP Assay Buffer	44	45.8	46	47.8
ATP Probe	2	0.2	2	0.2
ATP Converter	2	2	0	0
Developer Mix	2	2	2	2

1. Add 50 µL of Reaction Mix into each standard and sample wells.
2. Add 50 µL of Background Reaction Mix into the background control sample wells.

∆ **Note:** in absence of ATP converter, the assay detects only glycerol phosphate but not ATP.

3. Mix and incubate at room temperature for 30 min protected from light.
4. Measure output on a microplate reader at OD 570 nm.

∆ Note: we recommend measure reaction immediately but reaction is stable for at least 2 hours.

∆ Note: For fluorometric assays: The probe is very sensitive to exposure to air and light and hence should be kept tightly sealed, and should be added to the reaction mix right before the assay. In case of high background with samples and standards carefully inspect the probe color.

Calculations

Samples producing signals greater than that of the highest standard should be further diluted in appropriate buffer and reanalyzed, then multiply the concentration found by the appropriate dilution factor.

1. Average the duplicate reading for each standard, control and sample.

2. Subtract the mean value of the blank (Standard #1) from all standards, controls and sample readings. This is the corrected absorbance/relative fluorescence.

3. If significant, subtract the sample background control from sample readings.

4. Plot the corrected values for each standard as a function of the final concentration of ATP.

5. Draw the best smooth curve through these points to construct the standard curve. Most plate reader software or Excel can plot these values and curve fit. Calculate the trendline equation based on your standard curve data (use the equation that provides the most accurate fit).

6. Apply the corrected sample absorbance/relative fluorescence to the standard curve to get ATP (B) amount in the sample wells.

7. Concentration of ATP (nmol/µL or µmol/mL or mM) in the test samples is calculated as:

$$ATP\ concentration = \left(\frac{B}{V} * D\right) * DDF$$

Where:
B = amount of ATP in the sample well calculated from standard curve (nmol or mM).
V = sample volume added in the sample wells (µL).
D = sample dilution factor if sample is diluted to fit within the standard curve range (prior to reaction well set up)
DDF = deproteinization dilution factor (if alternative deproteinization step used).

ATP molecular weight = 507.18 g/mol

8. Using spiked samples, correct for any sample matrix interference by subtracting the sample reading from the spiked sample reading. This equation allows you to measure the ATP concentration in your sample when matrix interference is significant.

$$\mathrm{B} = \left(\frac{(ODsample\ corrected)}{(ODspiked\ corrected) - (ODsample\ corrected)}\right) * \mathrm{ATP\ Spike\ (nmol)}$$

Where:
B = ATP amount in sample well (nmol)
OD sample corrected = OD/RFU of sample with blank and background readings subtracted
OD spiked corrected = OD/RFU of spiked sample with blank and background readings subtracted
ATP Spike = amount of ATP spiked (nmol) into the sample well

Technical Support

For all technical or commercial enquiries please go to:
www.abcam.com/contactus
www.abcam.cn/contactus (China)
www.abcam.co.jp/contactus (Japan)

To get Chinese protocol, please scan QR code via WeChat or visit Protocols section at www.abcam.cn/ab83355.