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ab83355
ATP Assay Kit (Colorimetric/ Fluorometric)

For the rapid, sensitive and accurate measurement of ATP in a variety of samples.

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

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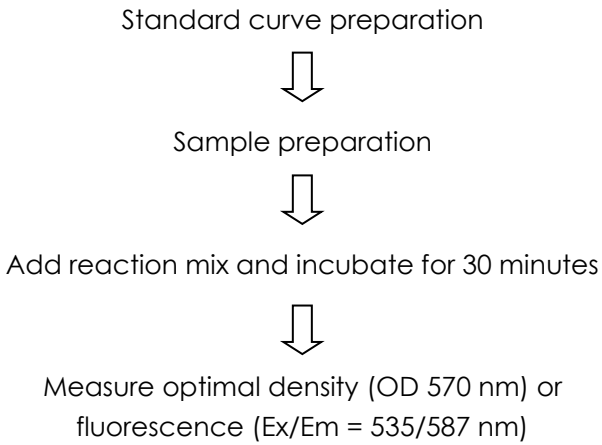
1. Overview

ATP Assay Kit (Colorimetric/ Fluorometric) (ab83355) is a robust, simple method to quantify total ATP in cell and tissue lysates, biological fluids and blood cells. The assay is based on the phosphorylation of glycerol in order to generate a product that can be easily quantified colorimetrically (OD 570 nm) or fluorometrically (Ex/Em = 535/587 nm).

Fluorometric assay is 10-100 fold more sensitive than colorimetric, and it can detect as low as 1 µM of ATP present in the samples.



2. Quick Assay Procedure

Δ Note: This procedure is provided as a quick reference for experienced users. Follow the detailed procedure when performing the assay for the first time.

- Prepare reagents and aliquot; get equipment ready.
- Prepare ATP Standard II dilution for your desired detection method: colorimetric [2 – 10 nmol/well] or fluorometric [0.2 – 1 nmol/well].
- Prepare samples (including deproteinization step) in optimal dilutions to fit standard curve readings.
- Set up plate in duplicate for standard (50 µL), samples (50 µL) and background sample control wells (50 µL).
- Prepare a master mix for ATP Reaction Mix and (if appropriate) a master mix for Background Reaction Mix:

Component	Col / Bckg Reaction Mix (µL)	Fluo/ Bckg Reaction Mix (µL)
Assay Buffer 23	44 / 46	45.8 / 47.8
OxiRed™ Probe	2 / 2	0.2 / 0.2
Converter Mix B	2 / 0	2 / 0
Developer Mix N	2 / 2	2 / 2

- Add 50 µL Reaction to standard and sample wells.
- Add 50 µL Background Reaction Mix to Sample Background control wells.
- Incubate plate at RT for 30 minutes protected from light.
- Measure plate at OD 570 nm for colorimetric assay or at Ex/Em= 535/587 nm for fluorometric assay.

3. Materials Supplied and Storage

Store kit at -20°C in the dark on receipt. Reconstituted components are stable for 2 months.

Aliquot components in working volumes before storing at the recommended temperature.

Avoid repeated freeze-thaws of reagents.

Item	Quantity	Storage temperature (before prep)	Storage temperature (after prep)
Assay Buffer 23	25 mL	-20°C	-20°C
OxiRed™ Probe	0.2 mL	-20°C	-20°C. Protect from light.
Converter Mix B	1 vial	-20°C	-20°C
Developer Mix N	1 vial	-20°C	-20°C
ATP IV	1 vial	-20°C	-20°C.

PLEASE NOTE: Assay Buffer 23 was previously labelled as Assay Buffer XXIII and ATP Assay Buffer, and Converter Mix B as Converter Enzyme I and ATP Converter, and OxiRed™ Probe as ATP Probe, and Developer Mix N as Developer IV and Developer Mix, and ATP IV as ATP Standard II and ATP Standard. The composition has not changed.

4. 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 flat bottom, preferably black (for fluorometric assay)
- Dounce homogenizer (if using tissue)
- Perchloric Acid (4N) and Potassium Hydroxide (2N): for PCA 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.

5. General guidelines, precautions, and troubleshooting

Please observe safe laboratory practice and consult the safety datasheet.

For general guidelines, precautions, limitations on the use of our assay kits and general assay troubleshooting tips, particularly for first time users, please consult our guide: www.abcam.com/assaykitguidelines

For typical data produced using the assay, please see the assay datasheet on our website.

If applicable, please refer to the current Safety Data Sheet (SDS) provided with this product for safety, handling, and disposal information. The most up to date and current versions are available on our website https://www.abcam.com/en-us.

6. Reagent Preparation

Briefly centrifuge small vials at low speed prior to opening.

6.1 Assay Buffer 23:

Ready to use as supplied. Equilibrate to room temperature before use.

6.2 ATP IV:

Reconstitute in 100 µL of ddH_2O to generate a 10 mM ATP IV stock solution. Keep on ice while in use. Aliquot standard so that you have enough to perform the desired number of assays.

The ATP IV is pure ATP with no additives.

6.3 OxiRed™ Probe:

Ready to use as supplied. Warm the DMSO solution at 37 °C until completely thawed (~ 5 minutes), vortex briefly to create a homogenous solution, and spin down briefly to collect any contents trapped in the cap or seal. Keep at room temperature during use and reseal promptly to minimize moisture exposure.

For multiple use aliquot and store at −20 °C, protected from light and moisture. Stable for up to 2 months after thawing (≤3 freeze–thaw cycles).

6.4 Converter Mix B:

Dissolve in 220 µL Assay Buffer 23. Keep on ice during the assay. Aliquot Converter Mix B so that you have enough volume to perform the desired number of assays.

6.5 Developer Mix N:

Dissolve in 220 µL Assay Buffer 23. Keep on ice during the assay. Aliquot Developer Mix N so that you have enough volume to perform the desired number of assays.

7. Standard Preparation

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

7.1 For the colorimetric assay:

Dilute 10 µl of the ATP IV stock solution with 90 µl of dH2O to generate 1 mM ATP, mix well. Add 0, 2, 4, 6, 8, 10 µl into a series of wells and adjust volume to 50 µl/well with Assay Buffer 23 to generate 0, 2, 4, 6, 8, 10 nmol/well of ATP.

7.2 For the fluorometric assay:

Further dilute the 1 mM ATP to 0.01- 0.1 mM with the dH2O before generating the standard curve as indicated for the colorimetric assay.

Δ Note: Detection sensitivity of fluorometric assay is 10-100 fold higher than colorimetric assay.

8. Sample Preparation

General sample information:

- We recommend performing several dilutions of your sample to ensure the readings are within the standard value range.
- We recommend that you use fresh samples for the most reproducible assay. If you cannot perform the assay at the same time, we suggest that you snap freeze your samples in liquid nitrogen upon extraction and store them immediately at -80°C. When you are ready to test your samples, thaw them on ice. Be aware however that this might affect the stability of your samples and the readings can be lower than expected. Avoid multiple freeze-thaws.
- The stated amount of Assay Buffer 23 is sufficient for 100 wells worth of reactions, not necessarily 100 sample extractions from 100 different tissue samples.

Δ Note: 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).

Δ Note: Theoretically, the kit can be used with living cells, Saccharomyces cerevisiae (yeast) or cell medium, the optimal standardized conditions however must be empirically determined.

8.1 Cell lysates:

1. Harvest the amount of cells necessary for each assay (initial recommendation: 1 x 10^6 cells).
2. Wash cells with cold PBS.
3. Resuspend cells in 100 µL of Assay Buffer 23.
4. Homogenize cells quickly by pipetting up and down a few times.
5. Centrifuge 5 minutes at 4°C at 13,000 *g* in a cold microcentrifuge to remove any insoluble material.
6. Collect supernatant and transfer to a new tube.
7. Keep on ice.
   Cells samples may contain enzymes that can interfere with the assay. Remove these enzymes from sample by using Perchloric Acid (4N) and Potassium Hydroxide (2N). See step 8.5.

8.2 Tissue lysates:

For highly metabolically active tissues such as muscle, we recommend preparing tissue samples directly in PCA following the alternative protocol described in Step 8.5. If you don't have access to PCA, then proceed with the instructions described below.

1. Harvest the amount of tissue necessary for each assay (initial recommendation: 10 mg).
2. Wash tissue in cold PBS.
3. Homogenize tissue in 100 µL of Assay Buffer 23 with a Dounce homogenizer (preferred for better assay reproducibility) or pestle sitting on ice, with 10-15 passes (some tissues require more passes).
4. Centrifuge sample for 2-5 minutes at 4°C at 13,000 *g* using a cold microcentrifuge to remove any insoluble material.
5. Collect supernatant and transfer to a new tube.
6. Keep on ice.

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

Use heparin when collecting plasma or serum. EDTA and other chelators should be avoided.

High concentrations of protein interfere with the assay. Fluid samples containing high levels of protein can be deproteinized with our 10 kD Spin Columns (ab93349) to deproteinize biological fluids. Add sample to the spin column, centrifuge at 13,000 x*g* for 10 min at 4°C. Collect the filtrate.

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).

8.4 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 Perchloric Acid (4N) and Potassium Hydroxide (2N). See step 8.5.

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

1. Homogenize sample in 100 µL of Assay Buffer 23.
2. Lyse cells by snap freeze-thaw cycles.
3. Centrifuge sample for 2 minutes at 4°C at 13,000 x*g* using a microcentrifuge to remove any insoluble material.
4. Collect supernatant and transfer to a clean tube.
5. Keep on ice.
   Remove interfering enzymes from sample by using Perchloric Acid (4N) and Potassium Hydroxide (2N). See step 8.5.
   Δ Note: Do not extend incubation times of 5 min when using PCA. Longer incubation times may result in loss of ATP.

6. Alternatively, you can perform a PCA/KOH deproteinization step following the protocol described in section 8.5.

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

8.5 Deproteinization protocol:

For this step you will need additional reagents:

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

Prepare samples as specified in the 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.

Δ Note: Do not extend incubation time. Longer incubation times may result in loss of ATP.

3. Centrifuge samples at 13,000 x*g* 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 50% 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 as needed.
6. Centrifuge at 13,000x *g* 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.

9. Assay Procedure – Colorimetric 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.

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.

9.1 Plate Loading (clear plate):

- Standard wells = 50 µL standard dilutions.
- Sample wells = 1-50 µL samples (adjust volume to 50 µL/well with Assay Buffer 23).
- Sample Background Control wells = 1-50 µL samples (adjust volume to 50µL/well with Assay Buffer 23).
  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.

9.2 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)
Assay Buffer 23	44	46
OxiRed™ Probe	2	2
Converter Mix B	2	0
Developer Mix N	2	2

2. Add 50 µL of Reaction Mix into each standard and sample wells.

3. Add 50 µL of Background Reaction Mix into the background control sample wells.

∆ **Note:** In absence of Converter Mix B, the assay detects only endogenous glycerol phosphate but not ATP.

4. Mix and incubate at room temperature for 30 min protected from light.
5. 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.

10. Assay Procedure – 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 the fluorometric assay, use 1/10 of the OxiRed™ Probe to reduce 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.

10.1 Plate Loading (black walled, clear bottom plate):

- Standard wells = 50 µL standard dilutions.
- Sample wells = 1-50 µL samples (adjust volume to 50 µL/well with Assay Buffer 23).
- Sample Background Control wells = 1-50 µL samples (adjust volume to 50µL/well with Assay Buffer 23).

10.2 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)
Assay Buffer 23	45.8	47.8
OxiRed™ Probe	0.2	0.2
Converter Mix B	2	0
Developer Mix N	2	2

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

Δ Note: In absence of Converter Mix B, the assay detects only endogenous glycerol phosphate but not ATP.

4. Mix and incubate at room temperature for 30 min protected from light.

5. Measure output on a microplate reader at Ex/Em = 535/587 nm.

Δ Note: We recommend measure reaction immediately but reaction is stable for at least 2 hours.

Δ Note: The OxiRed™ 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 OxiRed™ Probe color.

11. 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 (from Section 8.5).

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.

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

Where:

B = Amount of ATP in the sample well calculated from standard curve (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

12. Typical Data

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

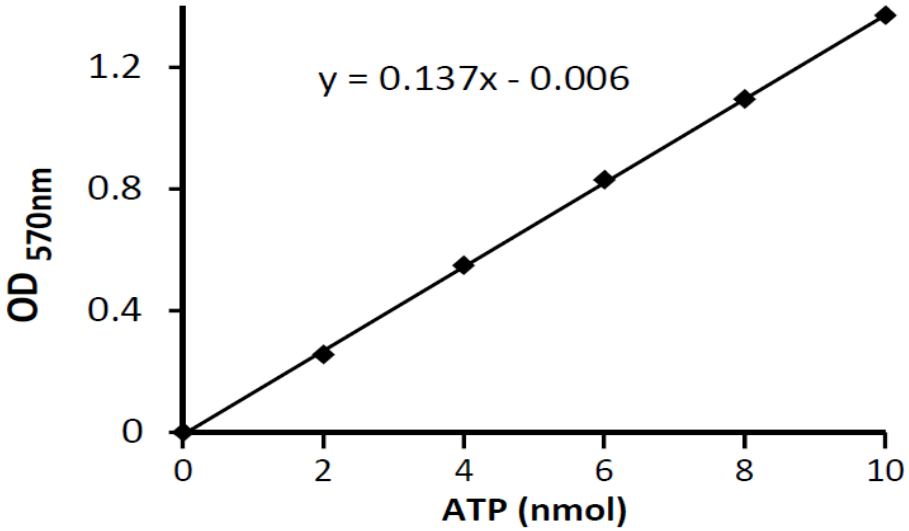


Figure 1. Typical ATP standard calibration curve using colorimetric reading.

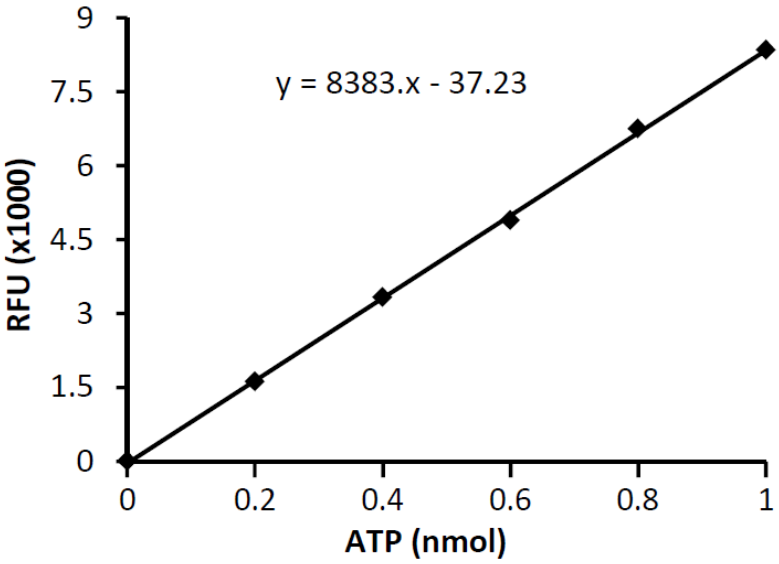


Figure 2. Typical ATP standard calibration curve using fluorometric reading.

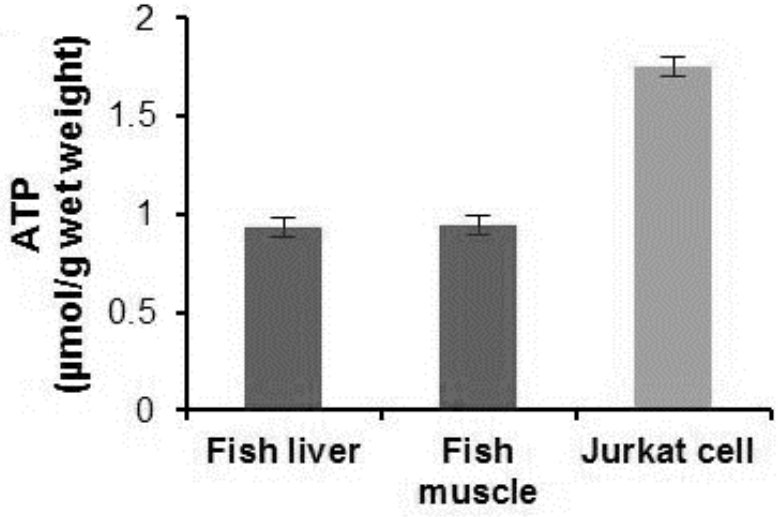


Figure 3. Quantitation of ATP in fish liver (2.5 µL of 10X diluted sample), fish muscle (5 µL of 10X diluted sample) and Jurkat cell lysate (5 µL) using fluorometric assay. Samples were spiked with known amounts of ATP (300 pmol).

13. Notes

Technical Support

For all technical or commercial enquiries please go to:

https://www.abcam.com/en-us/contact-us

https://www.abcam.cn/contact-us (China)

https://www.abcam.co.jp/contact-us (Japan)