



ELISA PRODUCT INFORMATION & MANUAL

ATP Assay Kit (Colorimetric/Fluorometric)

NBP2-54855

Enzyme-linked Immunosorbent Assay for quantitative detection. For research use only.

Not for diagnostic or therapeutic procedures.

www.novusbio.com - P: 303.730.1950 - P: 888.506.6887 - F: 303.730.1966 - technical@novusbio.com

Novus kits are guaranteed for 6 months from date of receipt

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1. Overview

ATP Assay Kit (Colorimetric/Fluorometric) (NBP2-54855) 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.

Standard curve preparation



Sample preparation



Add reaction mix and incubate for 30 minutes



Measure optimal density (OD 570 nm) or
fluorescence (Ex/Em = 535/587 nm)

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 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 μ L), samples (50 μ L) and background sample control wells (50 μ L).
- 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)
ATP Assay Buffer	44 / 46	45.8 / 47.8
ATP Probe	2 / 2	0.2 / 0.2
ATP Converter	2 / 0	2 / 0
Developer Mix	2 / 2	2 / 2

- Add 50 μ L Reaction to standard and sample wells.
- Add 50 μ L 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. Kit can be stored for 1 year from receipt, 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.

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.

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 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

5. General guidelines, precautions, and troubleshooting

Please observe safe laboratory practice and consult the safety datasheet.

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

6. Reagent Preparation

Briefly centrifuge small vials at low speed prior to opening.

6.1 ATP Assay Buffer:

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

6.2 ATP Standard (lyophilized, 1 μmol):

Reconstitute in 100 μL of ddH₂O to generate a 10 mM ATP standard stock solution. Keep on ice while in use. Aliquot standard so that you have enough to perform the desired number of assays.

The ATP standard is pure ATP with no additives.

6.3 ATP Probe (in DMSO):

Ready to use as supplied. Warm by placing in a 37°C bath for 1 – 5 min to thaw the DMSO solution before use.

Δ Note: DMSO tends to be solid when stored at -20°C, even when left at room temperature, so it needs to melt for a few minutes at 37°C. Repeat this step every time probe is needed.

Aliquot probe so that you have enough volume to perform the desired number of assays.

6.4 ATP Converter:

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

6.5 Developer Mix (lyophilized):

Dissolve in 220 μL ATP Assay Buffer. Keep on ice during the assay. Aliquot developer 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 Standard with 90 μl of dH₂O to generate 1 mM ATP standard, mix well. Add 0, 2, 4, 6, 8, 10 μl into a series of wells and adjust volume to 50 μl /well with ATP Assay Buffer to generate 0, 2, 4, 6, 8, 10 nmol/well of ATP Standard.

7.2 For the fluorometric assay:

Further dilute the ATP Standard 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 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: 1×10^6 cells).
2. Wash cells with cold PBS.
3. Resuspend cells in 100 μL of ATP Assay Buffer.
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.
8. Cells samples may contain enzymes that can interfere with the assay. Remove these enzymes from sample by using Deproteinizing Sample Preparation Kit – TCA (ab204708).

Alternatively, you can perform a PCA/KOH deproteinization step following the protocol described in section 8.6.

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 8.3. 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 μ L of ATP Assay Buffer 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.
7. Tissue samples may contain enzymes that can interfere with the assay. Remove these enzymes from sample by using Deproteinizing Sample Preparation Kit – TCA (ab204708). Alternatively, you can perform a PCA/KOH deproteinization step following the protocol described in section 8.3 or 8.6.

8.3 Highly metabolically active tissues – alternative protocol:

Δ 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).
2. Wash tissue in cold PBS.
3. Homogenize tissue in 100 μ L ice cold 2N PCA with a Dounce homogenizer sitting on ice, with 10-15 passes.
4. Keep samples on ice for 30-45 minutes.
5. 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.
6. After homogenizing the tissue in 100 μ L of PCA, dilute the volume to 500 μ L with the ATP Assay Buffer.

7. Precipitate excess PCA by adding ice-cold 2M KOH that equals 10-20% of the total volume (for instance, 50-100 μL of 2 M KOH to 500 μL sample) and vortexing briefly. This will neutralize the sample and precipitate excess PCA.
8. 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.
9. Centrifuge at 13,000 g for 15 minutes at 4°C and collect supernatant.
10. 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.
11. To calculate the dilution factor introduced by the deproteinization step (DDF), simply apply the following formula:

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

Δ Note: 500 μL is final sample volume (PCA + Assay Buffer) before neutralization. Initial sample volume in PCA will be 100 μL .

8.4 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 xg for 10 min at 4°C. Collect the filtrate.

To 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).

8.5 Red blood cells (RBC):

We recommend harvesting 1×10^7 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 μ L 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 in section 8.6.

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

8.6 Alternative 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.
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 μ L 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:

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

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

9.2 ATP reaction mix:

1. Prepare 50 μ L 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 (μ L)	Background Reaction Mix (μ L)
ATP Assay Buffer	44	46
ATP Probe	2	2
ATP Converter	2	0
Developer Mix	2	2

2. Add 50 μ L 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 ATP converter, 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.

Δ 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 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.

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

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

10.2 ATP reaction mix:

1. Prepare 50 μ L 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 (μ L)	Background Reaction Mix (μ L)
ATP Assay Buffer	45.8	47.8
ATP Probe	0.2	0.2
ATP Converter	2	0
Developer Mix	2	2

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

Δ Note: In absence of ATP converter, 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 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.

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/ μ L or μ mol/mL or mM) in the test samples is calculated as:

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

Where:

B = amount of ATP in the sample well calculated from standard curve (nmol or mM).

V = sample volume added in the sample wells (μ L).

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 8.3 or 8.6).

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.

$$B = \left(\frac{(OD_{sample\ corrected})}{((OD_{spiked\ corrected}) - (OD_{sample\ corrected}))} \right) * ATP\ Spike\ (nmol)$$

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

12. Typical Data

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

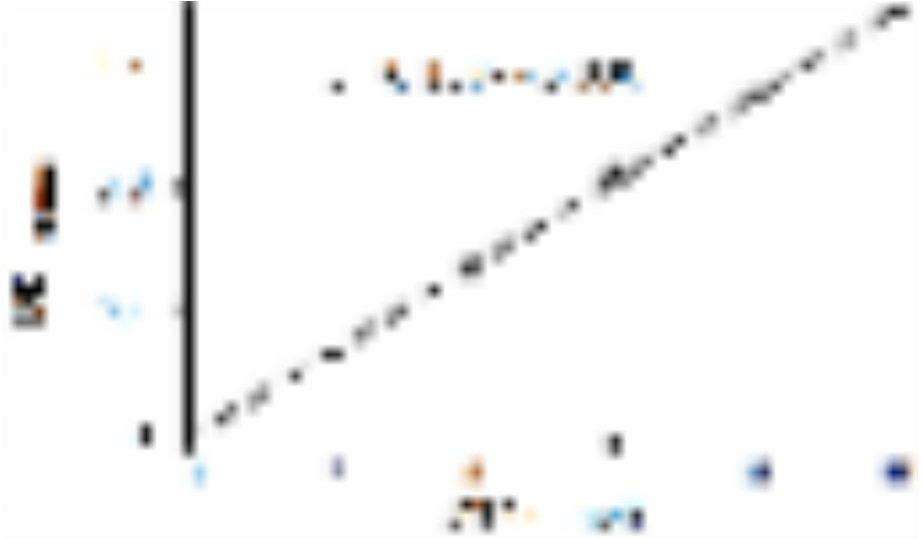


Figure 1. Typical ATP standard calibration curve using colorimetric reading.

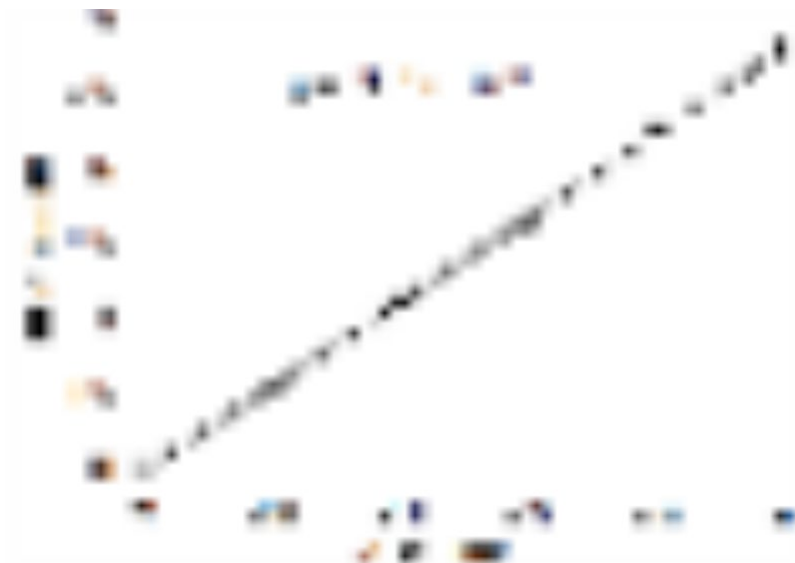


Figure 2. Typical ATP standard calibration curve using fluorometric reading.



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

