**Signosis, Inc.**
Innovative Plate Assay Solutions

# ATP Assay Kit

**Catalog # EA-7017** **(For Research Use Only)**

## Introduction

The ATP Assay Kit utilizes a couple of enzymes which catalyze the metabolism of glycerol to measure ATP levels in samples. First, glycerol kinase is used to convert glycerol to glycerol 3-phosphate. This step consumes ATP so the end product of this enzyme reaction can be used to quantify the amount of ATP present in the sample. In the next step, glycerol 3-phosphate oxidase reacts with glycerol 3-phosphate to produce hydrogen peroxide. The ATP level in the sample is determined by quantifying the hydrogen peroxide generated by the enzyme reaction with a fluorogenic probe that can be measured with a spectrophotometer.

## Materials Required but Not Provided

- PBS
- 96-well clear microplate for absorbance reading or 96-well black microplate with clear bottom for fluorescence reading
- Microplate reader capable of measuring absorbance at 560 nm or fluorescence at 530nm/590nm

## Materials Provided

- ATP Buffer (RT)
- Probe Reagent (-20ºC)
- HRP Reagent (4ºC)
- 1mM ATP Standard (-80ºC)
- GK Enzyme Stock (-80ºC)
- GPO Enzyme Stock (-80ºC)

****Spin down small tubes before starting experiment. ****

**Plasma Sample Preparation**

1. Centrifuge citrated or EDTA-collected blood at 4°C (1,000 x *g* for 10 minutes) to separate plasma from erythrocytes. Alternatively, blood collected without anticoagulant can be centrifuged to collect serum.
2. Transfer the plasma layer to a new tube without disturbing the buffy layer.
3. The plasma may be assayed directly or stored away at -80°C.

**Cell Sample Preparation**

1. Wash the cells once with PBS before lysing the cells.
2. For a 96-well culture plate, add 40 µL of Lysis buffer to each well and incubate at room temperature for 10 minutes.
3. Pipette the Lysis buffer up and down to detach the cells and transfer the cell lysates into a new tube.
4. If necessary, homogenize the cell lysates with a sonicator.
5. The cell lysates may be assayed directly or stored at -80°C.

**Tissue Sample Preparation**

1. Weigh tissue sample and add 1 mL of Tissue Lysis buffer per 100mg of tissue.
2. Homogenize the tissue samples with a tissue grinder.
3. If necessary, further homogenize the tissue samples with a sonicator.
4. Centrifuge the sample at 10,000 RPM for 5 minutes to pellet the tissue debris.
5. Collect the supernatant and measure the protein concentration of the supernatant. The tissue sample can be assayed directly or stored at -80°C.
6. Use the Dilution buffer to dilute the tissue sample to the appropriate concentration for each assay.

**ATP Measurement**

1. Standard curve preparation: Using the provided 1mM ATP standard, prepare a standard curve dilution as described in the table below

| Standard # | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
|---|---|---|---|---|---|---|---|---|
| ATP Standard Volume (µL) | 10 | 8 | 6 | 4 | 2 | 1 | 0.5 | 0 |
| PBS (µL) | 40 | 42 | 44 | 46 | 48 | 49 | 49.5 | 50 |
| Total (µL) | 50 | 50 | 50 | 50 | 50 | 50 | 50 | 50 |
| ATP Final Concentration (µM) | 200 | 160 | 120 | 80 | 40 | 20 | 10 | 0 |

2. Reaction mix preparation: calculate the amount of each reagent needed to make the reaction mix according to the table below.

| Component | Reaction Mix (per well/sample) |
|---|---|
| GK Enzyme | 0.04 µL |
| GPO Enzyme | 0.04 µL |
| ATP Buffer | 39.92 µL |
| Total | 40 µL |

3. Any unused enzyme stock can be stored at -80°C for future use.
4. Add 40 µL of reaction mix to each well of the plate.
5. Add 40 µL of sample or standard to each well with reaction mix and mix thoroughly.
6. Cover the plate and incubate at room temperature for 30 minutes.
7. Detection mix preparation: calculate the amount of each reagent needed to make the detection mix according to the table below.

| Component | Detection Mix (per well/sample) |
|---|---|
| Probe Reagent | 0.8 µL |
| HRP Reagent | 1.6 µL |
| PBS | 77.6 µL |
| Total | 80 µL |

8. Add 80 µL of detection mix to each sample or standard well in the plate. **Be sure to add the detection mix quickly, since the signal begins to develop when the reagents are added. Use a multichannel pipette if possible.**
9. Cover the plate and incubate at room temperature away from light for 15-30 minutes. **Exposure to light will produce background signal in wells**
10. For a stronger signal, the plate can be incubated for another 30-60 minutes away from light.
11. Measure the absorbance of the plate at 560 nm using a plate reader. Alternatively, measure the fluorescence of the plate in a fluorescence plate reader Ex/Em 530nm/590nm.