Signosis, Inc.
Innovative Plate Assay Solutions

NAD+/NADH Assay Kit

Catalog # EA-7018 **(For Research Use Only)**

Introduction

The NADH Assay utilizes WST-8, a water-soluble dye, to measure NADH levels in samples. WST-8 reacts with NADH to produce a yellow color, which can be measured at an absorbance of 450 nm with a plate reader. Total NAD+ and NADH levels can be detected by converting NAD+ to NADH with alcohol dehydrogenase.

Materials Required but Not Provided

- PBS
- 96-well clear microplate for absorbance reading
- Microplate reader capable of measuring absorbance at 450 nm

Materials Provided

- ADH Buffer (RT)
- 1mM NADH Standard (-20ºC)
- NADH Buffer (4ºC)
- 200x ADH Enzyme Stock (-80ºC)
- Mediator Reagent (-20ºC)
- WST Reagent (-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.
6. Use PBS to dilute the cell sample to the appropriate concentration, if necessary.

Tissue Sample Preparation

1. Weigh tissue sample and add 1 mL of 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 PBS to dilute the tissue sample to the appropriate concentration, if necessary.

NADH Measurement

1. Standard curve preparation: First, take eight new tubes and label them 1-8. In tube 1, add 120 µL of the provided 1mM NADH standard. Then, for tubes 2-8, add 60 µL of PBS to each tube. Transfer 60 µL of the 1mM NADH standard from tube 1 to the PBS in tube 2 and mix to make a 500µM NADH standard. Then, transfer 60 µL of the 500µM NADH standard from tube 2 to the PBS in tube 3 and mix to make an 250µM NADH standard. Continue the serial dilutions until tube 7 is done. Leave tube 8 untouched as the PBS negative control.

Standard#	NADH Concentration (µM)
1	1000
2	500
3	250
4	125
5	62.5
6	31.25
7	15.625
8	0

2. Add 50 µL of sample or standard to each well.
3. 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)
WST Reagent	5 µL
Mediator	0.5 µL
PBS	44.5 µL
Total	50 µL

4. Add 50 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.**
5. Cover the plate and incubate at 37°C away from light for 15-30 minutes.
   Exposure to light will produce background signal in wells
6. For a stronger signal, the plate can be incubated for another 30-60 minutes at 37°C away from light.
7. Measure the absorbance of the plate at 450 nm using a plate reader.

NAD+ and NADH Total Measurement

1. 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)
1x ADH Enzyme	5 µL
ADH Buffer	45 µL
Total	50 µL

2. Dilute 200x ADH enzyme stock to 1x in PBS for the assay. Any unused enzyme stock can be stored at -80°C for future use.
3. Add 50 µL of reaction mix to each well of the plate.
4. Add 5 µL of sample or standard to each well with reaction mix and mix thoroughly.
5. Cover the plate and incubate at 37°C for 10 minutes.
6. 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)
WST Reagent	5 µL
Mediator Reagent	0.5 µL
PBS	44.5 µL
Total	50 µL

7. Add 50 µL of detection mix to each sample or standard well in the plate.
8. Cover the plate and incubate at 37°C away from light for 30-60 minutes.
   Exposure to light will produce background signal in wells
9. For a stronger signal, the plate can be incubated for an additional 1-2 hours at 37°C away from light.
10. Measure the absorbance of the plate at 450 nm using a plate reader.