# TECHNICAL MANUAL

# NADPH Oxidase (NAO) Activity Colorimetric Assay Kit

- **SKU CODE:** MAES0522
- **SIZE:** 48 Tests
- **DETECTION PRINCIPLE:** Assay Kit
- **RUO:** Research-Use-Only

Revised: 01/2026

# 1. Assay summary

- Reagent preparation
- Sample preparation
- Operating steps
- Calculation

# 2. Intended use

This kit can be used to measure NADPH oxidase (NAO) activity in cells, animal and plant tissue samples.

# 3. Detection principle

NADPH Oxidase (NAO) is a membrane protein mainly found in mammalian neutrophils, plant cells and filamentous fungal cells, and was initially discovered in phagocytic cell membranes. NAO can oxidize NADPH to NAD+ while generating superoxide anions, and it is one of the main sources of reactive oxygen species (ROS) in the body. NAO is involved in many important physiological processes, including host defense, post-translational modification of proteins, cell signal transduction, gene expression regulation and cell differentiation.

The detection principle of this kit is as follows: NAO catalyzes the substrate to produce product, which react with chromogenic agent to form chromogenic substance. It has the maximum absorption at 450 nm. The enzyme activity of NAO is calculated by measuring the OD value at 450 nm.

# 4. Kit components & storage

| Item | Component | Size 1(48 T) | Size 2(96 T) | Storage |
|---|---|---|---|---|
| Reagent 1 | Extraction Solution | 25 mL x 1 vial | 50 mL x 1 vial | -20 °C, 12 months |
| Reagent 2 | Buffer Solution | 11 mL x 1 vial | 22 mL x 1 vial | -20 °C, 12 months, shading light |
| Reagent 3 | Inhibitor | 0.3 mL x 1 vial | 0.6 mL x 1 vial | -20 °C, 12 months, shading light |

| Item | Component | Size 1(48 T) | Size 2(96 T) | Storage |
|---|---|---|---|---|
| Reagent 4 | Substrate | Powder x 1 vial | Powder x 2 vials | -20 °C, 12 months, shading light |
| Reagent 5 | Chromogenic Agent | 1.5 mL x 1 vial | 3 mL x 1 vial | -20 °C, 12 months, shading light |
| | Microplate | 48 wells | 96 wells | No requirement |
| | Plate Sealer | 2 pieces | 2 pieces | |

# 5. Materials prepared by users

**Instruments:**

Microplate reader (440-460 nm, optimum wavelength: 450 nm), Incubator

# 6. Reagent preparation

1. Equilibrate all reagents to 25 °C before use.
2. **Preparation of substrate working solution:** Dissolve one vial of substrate with 550 µL of double distilled water, mix well to dissolve. Store at -20 °C for a week protected from light.
3. **Preparation of working solution:** For each well, prepare 80 µL of working solution (mix well 45 µL of buffer solution, 10 µL of substrate working solution and 25 µL of chromogenic agent). The working solution should be prepared on spot protected from light and used up within 30 min.

# 7. Sample preparation

Sample preparation

**Tissue sample:**

1. Harvest the amount of tissue needed for each assay (initial recommendation 20 mg).

2. Homogenize 20 mg tissue in 180 µL extraction solution with a dounce homogenizer at 4 °C.

3. Centrifuge at 10000 xg for 10 min at 4 °C to remove insoluble material. Collect supernatant and keep it on ice for detection. The supernatant should be used on the same day.

**Cell samples:**

1. Harvest the number of cells needed for each assay (initial recommendation 1 x $10^6$ cells).

2. Homogenize 1 x $10^6$ cells in 200 µL extraction solution with an ultrasonic cell disruptor at 4 °C.

3. Centrifuge at 10000 xg for 10 min at 4 °C to remove insoluble material. Collect supernatant and keep it on ice for detection. The supernatant should be used on the same day.

Dilution of sample

The recommended dilution factor for different samples is as follows (for reference only):

Sample type - Dilution factor

10% Mouse liver tissue homogenate - 2-10

10% Mouse spleen tissue homogenate - 1

10% Mouse heart tissue homogenate - 1

10% Mouse brain tissue homogenate - 1

10% Mouse kidney tissue homogenate - 1

10% Mouse lung tissue homogenate - 1

10% Potato tissue homogenate - 1

10% Corn tissue homogenate - 1

10% Garlic tissue homogenate - 1

10% Broccoli tissue homogenate - 1

1 x $10^6$ HeLa cells - 1

1 x $10^6$ Jurkat cells - 1

1 x $10^6$ RAW 264.7 cells - 1

**Note:** The diluent is extraction solution. For the dilution of other sample types, please perform a pretest to confirm the dilution factor.

# 8. The key points of the assay

1. Equilibrate Inhibitor to 25 °C before use. On operating step 2, add 10 µL of inhibitor into control wells. It is not allowed to mix inhibitor and buffer solution together when adding, otherwise, a large amount of precipitate will be produced.
2. The control wells may have a small amount of precipitation that does not affect the experimental results.

# 9. Operating steps

1. Sample well: Add 20 µL of samples into wells. Control well: Add 20 µL of samples into wells.
2. Add 10 µL of inhibitor into control wells.
3. Add 100 µL of buffer solution into sample wells. Add 90 µL of buffer solution into control wells.
4. Mix fully for 5 s with microplate reader and incubate at 37 °C for 10 min.
5. Add 80 µL of working solution into each well.
6. Mix fully for 5 s with microplate reader and measure the OD value of each well at 450 nm, as A1. Incubate at 37 °C for 10 min and measure the OD value of each well at 450 nm, as A2.

# 10. Calculation

**The sample:**

1. Tissue sample:

**Definition:** The amount of enzyme in 1 kg tissue samples per 1 min that catalyze the production of 1 µmol of product from the substrate at 37 °C is defined as 1 unit.

NAO activity (U/kg wet weight) = $(deltaA_{sample} - deltaA_{control}) / (\varepsilon \times d) \times (V_1/V_2) \div (m/V) \div T \times f \times 1000$

2. Cell sample:

**Definition:** The amount of enzyme in 1 x $10^9$ cells samples per 1 min that catalyze the production of 1 nmol of product from the substrate at 37 °C is defined as 1 unit.

NAO activity ($U/10^9$) = $(deltaA_{sample} - deltaA_{control}) / (\varepsilon \times d) \times (V_1/V_2) \div (n/V_3) \div T \times f \times 1000$

[Note]

$deltaA_{sample}$: $deltaA_{sample} = A_2 – A_1$.

$deltaA_{control}$: $deltaA_{control} = A_2 – A_1$.

ε: The molar extinction coefficient of chromogenic substance at 450 nm, 30.7 L/mmol/cm.

d: Optical path, 0.6 cm.

$V_1$: The volume of reaction system, µL.

$V_2$: The volume of sample added to the reaction system, µL.

m: The wet weight of sample, kg.

V: The volume of extraction solution in the preparation step of tissue, L.

T: Reaction time, 10 min.

n: The number of cell sample/$10^6$.

$V_3$: The volume of extraction solution in the preparation step of cell, L.

f: Dilution factor of sample before test.

1000: 1 mmol/L = 1000 μmol/L

# 11. Performance Characteristics

Parameter:

## Intra-assay Precision

Three mouse heart tissue samples were assayed in replicates of 20 to determine precision within an assay (CV = Coefficient of Variation).

## Intra-assay Precision

| Parameters | Sample 1 | Sample 2 | Sample 3 |
|---|---|---|---|
| Mean (U/L) | 5.00 | 15.00 | 30.00 |
| %CV | 2.7 | 4.1 | 3.5 |

## Inter-assay Precision

Three mouse heart tissue samples were assayed 20 times in duplicate by three operators to determine precision between assays.

## Inter-assay Precision

| Parameters | Sample 1 | Sample 2 | Sample 3 |
|---|---|---|---|
| Mean (U/L) | 5.00 | 15.00 | 30.00 |
| %CV | 5.0 | 9.5 | 7.6 |

## Recovery

Take three samples of high concentration, middle concentration and low concentration to test the samples of each concentration for 6 times in parallel to get the average recovery rate of 100%.

## Recovery

| Sample | Expected Conc. (U/L) | Observed Conc. (U/L) | Recovery rate (%) |
|---|---|---|---|
| Sample 1 | 2 | 1.90 | 95 |
| Sample 2 | 10 | 10.30 | 103 |
| Sample 3 | 20 | 20.40 | 102 |

## Sensitivity

The analytical sensitivity of the assay is 0.27 U/L. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 20 times, and calculating the corresponding concentration.

# 12. Example Analysis

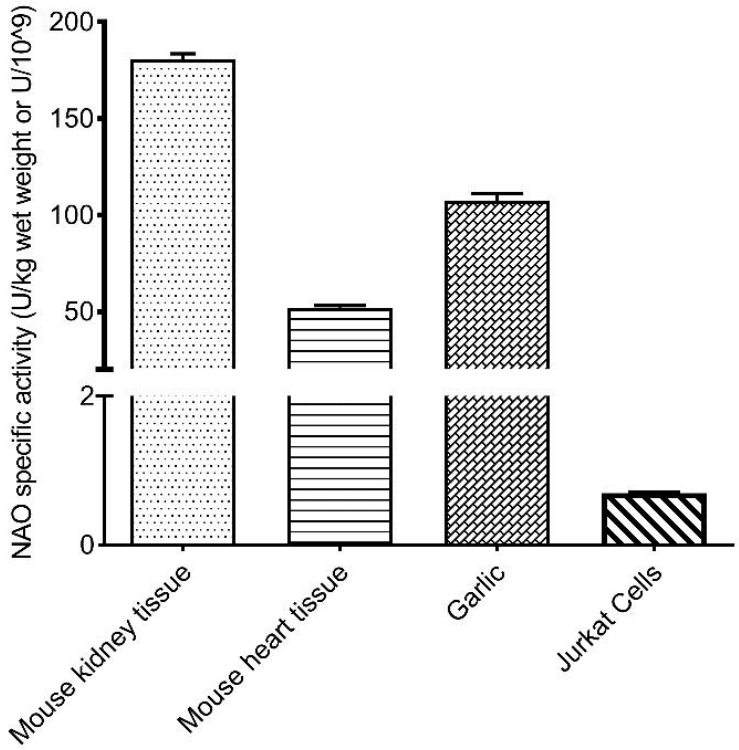
**Example analysis:**

Take 20 µL of 10% mouse kidney tissue homogenate which dilute 2 times and carry the assay according to the operation steps. The results are as follows:

The $A_1$ of the sample well is 0.171, the $A_2$ of the sample well is 0.379, $deltaA_{sample} = A_2 - A_1$ = 0.379 - 0.171 = 0.208, The $A_1$ of the control well is 0.202, the $A_2$ of the control well is 0.225, $deltaA_{control} = A_2 - A_1$ = 0.225 - 0.202 = 0.023, and the calculation result is:

NAO activity (U/kg wet weight) = (0.208 - 0.023) ÷ (30.7 × 0.6) × (200 ÷ 20) ÷ (0.0001 ÷ 0.0009) ÷ 10 × 2× 1000 = 180.78 U/kg wet weight

Detect 10% mouse kidney tissue homogenate (dilute 2 times), 10% mouse heart tissue homogenate, 10% garlic tissue homogenate and $2\times10^6$ Jurkat cells, according to the protocol, the result is as follows:




## 13. Statement

**1.** This assay kit is for Research Use Only. Assay Genie assumes no responsibility for any problems or legal liabilities arising from the use of this kit for clinical diagnosis or any other purpose.

**2.** Please read the instructions carefully and calibrate the instruments before performing the experiments. Follow the instructions strictly throughout the procedure.

**3.** Appropriate protective measures must be taken, including wearing a lab coat and latex gloves.

**4.** If the concentration of the substance falls outside the detection range, perform an additional dilution or concentration step on the sample.

**5.** It is recommended to perform a pre-test if your sample type is not listed in the instruction manual.

**6.** Experimental results are closely related to reagent quality, operator technique, environmental conditions, and other factors. Assay Genie guarantees the quality of the kits only and is NOT responsible for sample consumption resulting from use of the assay kits. It is advisable to estimate the expected sample usage and reserve sufficient samples before starting the experiment.

**Assay Genie 100% money-back guarantee!**

If you are not satisfied with the quality of our products and our technical team cannot resolve your problem, we will give you 100% of your money back.

**Manufacturers Statement: This final kit system is assembled and quality-released by Assay Genie Limited.**