# Product Information & Manual

## Total Antioxidant Status/TAS Assay Kit (Colorimetric)

NBP3-25821

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Novus kits are guaranteed for 6 months from date of receipt.

**For research use only. Not for diagnostic or therapeutic procedures.**

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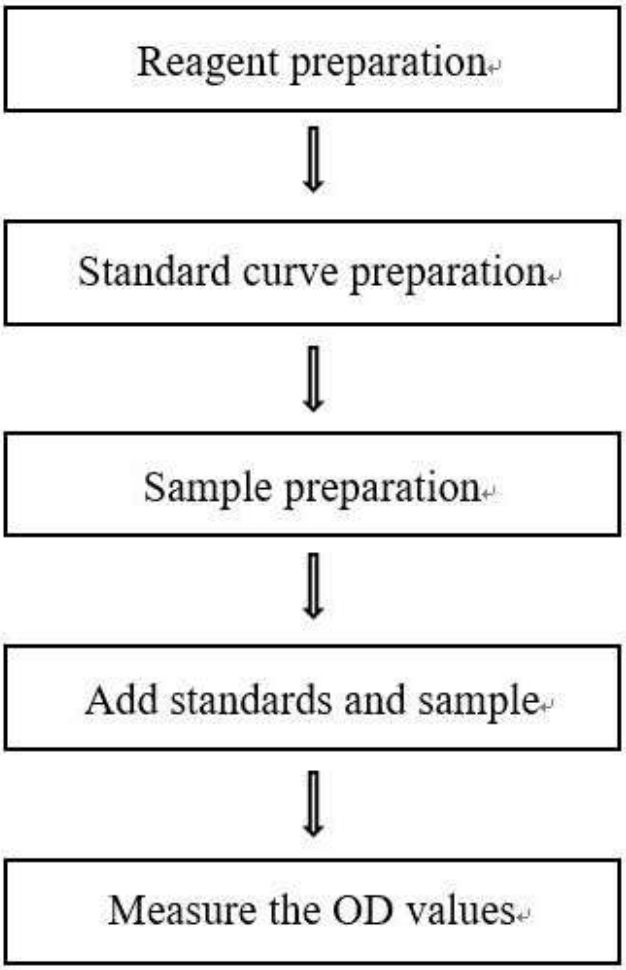
## Assay summary



## Intended use

The kit is used for the determination of Total Antioxidant Status (TAS) in serum, plasma, urine, cellular supernatant, animal and plant tissue samples.

## Detection principle

ABTS is oxidized to green ABTS•+ by appropriate oxidant, which can be reduced to colorless ABTS in the presence of antioxidants. The TAS of the sample can be determined and calculated by measuring the absorbance of $ABTS^{+}$ at 660 nm. Trolox is an analog of VE and has a similar antioxidant state to that of VE. Trolox is used as a reference substance for total antioxidant status.

## Kit components & storage

| Item | Component | Size | Storage |
|---|---|---|---|
| Reagent 1 | Buffer Solution | 30 mL × 1 vial | -20℃, 12 months |
| Reagent 2 | Chromogenic Agent | 5 mL × 1 vial | -20℃, 12 months, shading light |
| Reagent 3 | 2 mmol/L Standard | 4 mL × 1 vial | -20℃, 12 months, shading light |
| | Microplate | 96 wells | No requirement |
| | Plate Sealer | 2 pieces | |

Note: The reagents must be stored strictly according to the preservation conditions in the above table. The reagents in different kits cannot be mixed with each other. For a small volume of reagents, please centrifuge before use, so as not to obtain sufficient amount of reagents.

## Materials prepared by users

**Instruments:**

Microplate reader (650-670 nm, optimum wavelength: 660 nm), Micropipettor, 37°C incubator

**Reagents:**

Double distilled water, 60% Ethanol

## Reagent preparation

① Equilibrate all reagents to room temperature before use.

② The preparation of standard curve：

Always prepare a fresh set of standards. Discard working standard dilutions after use.

Dilute 2 mmol/L standard solution with 60% ethanol to a serial concentration. The recommended dilution gradient is as follows: 0, 0.4, 0.8, 1.2, 1.4, 1.6, 1.8, 2 mmol/L. Reference is as follows:

| Item | ① | ② | ③ | ④ | ⑤ | ⑥ | ⑦ | ⑧ |
|---|---|---|---|---|---|---|---|---|
| **Concentration (mmol/L)** | **0** | **0.4** | **0.8** | **1.2** | **1.4** | **1.6** | **1.8** | **2** |
| **2 mmol/L standard (μL)** | 0 | 40 | 80 | 120 | 140 | 160 | 180 | 200 |
| **60% ethanol (μL)** | 200 | 160 | 120 | 80 | 60 | 40 | 20 | 0 |

## Sample preparation

### ① Sample preparation

**Serum and plasma:** detect directly. If not detected on the same day, the serum or plasma can be stored at -80℃ for a month.

**Tissue sample:**

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

② Wash tissue in cold PBS (0.01 M, pH 7.4).

③ Homogenize 20 mg tissue in 180 μL 60% ethanol with a dounce homogenizer at 4℃.

④ Centrifuge at 10000×g for 10 minutes at 4℃ to remove insoluble material. Collect supernatant and keep it on ice for detection.

### ② 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 | 1 |
| 10% Rat liver tissue homogenate | 1 |
| 10% Rat lung tissue homogenate | 1 |
| Molt4 cellar supernatant | 1 |
| Human urine | 8-10 |
| Mouse serum | 1 |
| Human serum | 1 |
| Human saliva | 1 |

Note: The diluent is 60% Ethanol. For the dilution of other sample types, please do pretest to confirm the dilution factor

## The key points of the assay

① When adding chromogenic agent, suck and beat with micropipettor repeatedly to ensure the color system mix fully.

② Avoid bubbles when adding samples.

## Operating steps

① Standard well: Add 10 μL of standard with different concentration to the standard well.

Sample well: Add 10 μL of sample to the sample well.

② Add 200 μL of buffer solution to each well.

③ Measure the OD values of each well at 660 nm with microplate reader, recorded as $A_1$.

④ Add 20 μL of chromogenic agent to each well, repeatedly suck and beat for 5-6 times.

⑤ Incubate at 37°C for 5 min. Measure the OD values of each well at 660 nm with microplate reader, recorded as $A_2$. $\Delta A = A_2 - A_1$.

## Calculation

**The standard curve**:

1. Average the duplicate reading for each standard.

2. Subtract the mean ΔA value of all standard from the blank (Standard #①). This is the absoluted ΔA value.

3. Plot the standard curve by using absoluted ΔA value of standard and correspondent concentration as y-axis and x-axis respectively. Create the standard curve (**y = ax + b**) with graph software (or EXCEL).

**The sample**:

1. Liquid sample (Trolox is used as a reference substance for total antioxidant status):

$$\text{TAS (mmol Trolox Equiv. /L)} = (\Delta A_{Blank} - \Delta A_{Sample} - b) \div a \times f$$

2. Tissue sample:

$$\text{TAS (mmol Trolox Equiv. /kg wet weight)} = (\Delta A_{Blank} - \Delta A_{Sample} - b) \div a \div (m \div v) \times f$$

**[Note]**

y: $\Delta A_{Blank} - \Delta A_{Standard}$ ($\Delta A_{Blank}$ is ΔA when the standard concentration is 0).

x: The concentration of Standard.

a: The slope of standard curve.

b: The intercept of standard curve.

$\Delta A_{Sample}$: The OD value of sample ($A_2$ - $A_1$).

m：The weight of tissue sample (g).

V：The volume of added homogenate (mL).

f: Dilution factor of sample before test.

# Appendix I Performance Characteristics

## 1. Parameter:

### Intra-assay Precision

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

| Parameters | Sample 1 | Sample 2 | Sample 3 |
|---|---|---|---|
| **Mean (mmol Trolox Equiv./L)** | 0.87 | 1.05 | 1.46 |
| **%CV** | 4.8 | 4.5 | 4.5 |

### Inter-assay Precision

Three human serum samples were assayed 20 times in duplicate by three operators to determine precision between assays.

| Parameters | Sample 1 | Sample 2 | Sample 3 |
|---|---|---|---|
| **Mean (mmol Trolox Equiv./L)** | 0.87 | 1.05 | 1.46 |
| **%CV** | 6.8 | 7.3 | 6.9 |

### Recovery

Take three samples of high concentration, middle concentration and low concentration to test the samples of each concentration for 6 times parallelly to get the average recovery rate of 99%.

| | Standard 1 | Standard 2 | Standard 3 |
|---|---|---|---|
| **Expected Conc. (mmol/L)** | 0.7 | 1.35 | 1.75 |
| **Observed Conc. (mmol/L)** | 0.7 | 1.3 | 1.7 |
| **Recovery rate(%)** | 101 | 99 | 97 |

**Detection range:** 0.23-2 mmol Trolox Equiv. /L

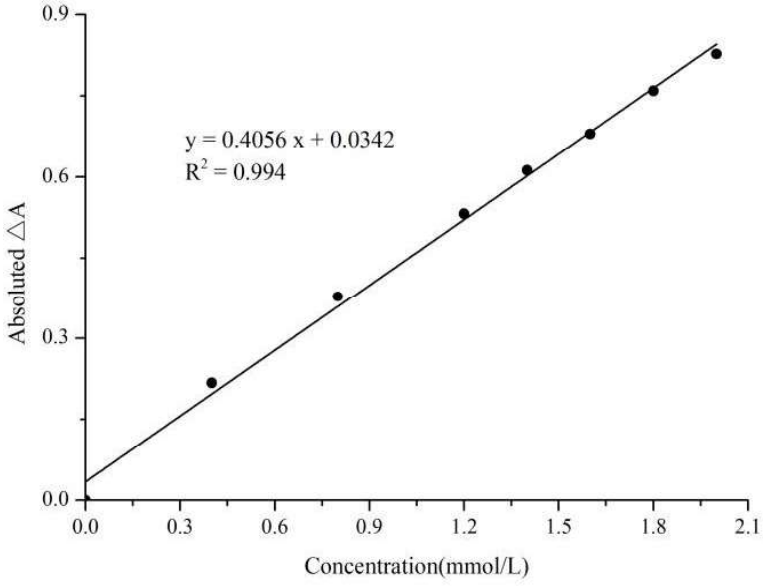
### Sensitivity

The analytical sensitivity of the assay is 0.23 mmol Trolox Equiv./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.

## 2. Standard curve:

As the ΔA value of the standard curve may vary according to the conditions of the actual assay performance (e.g. operator, pipetting technique or temperature effects), so the standard curve and data are provided as below for reference only：

| **Concentration (mmol/L)** | **0** | **0.4** | **0.8** | **1.2** | **1.4** | **1.6** | **1.8** | **2** |
|---|---|---|---|---|---|---|---|---|
| **Average ΔA** | 1.196 | 0.978 | 0.818 | 0.664 | 0.583 | 0.516 | 0.436 | 0.368 |
| **Absoluted ΔA** | 0.000 | 0.217 | 0.378 | 0.532 | 0.612 | 0.680 | 0.759 | 0.827 |



# Appendix Π Example Analysis

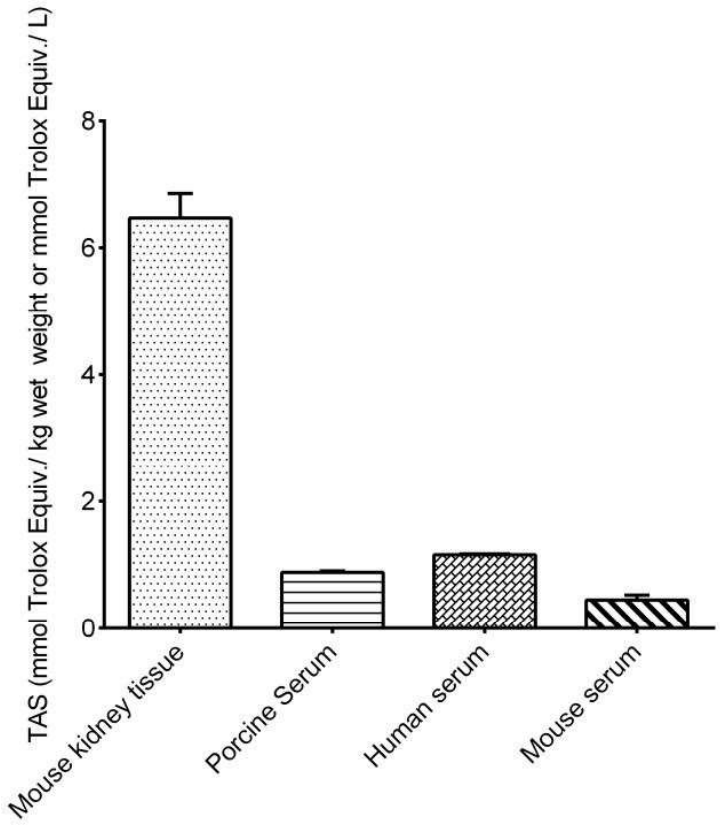
**Example analysis：**

For human serum, take human serum sample and carry the assay according to the operation steps. The results are as follows:

Standard curve: y = 0.4056 x + 0.0342, the OD value of the sample ($A_1$) is 0.08, the OD value of the sample ($A_2$) is 0.777, $\Delta A_{Sample} = A_2 - A_1 = 0.697$, $\Delta A_{Blank}$ is 1.196, and the calculation result is:

TAS (mmol Trolox Equiv. /L) = (1.196–0.697 -0.0342) ÷ 0.4056 =1.14 mmol Trolox Equiv. /L

Detect 10% Mouse kidney tissue homogenate (dilute for 4 times), human serum, mouse serum and porcine serum according to the protocol, the result is as follows：



# Statement

1. This assay kit is for Research Use Only. We will not response for any arising problems or legal responsibilities causing by using the kit for clinical diagnosis or other purpose.
2. Please read the instructions carefully and adjust the instruments before the experiments. Please follow the instructions strictly during the experiments.
3. Protection methods must be taken by wearing lab coat and latex gloves.
4. If the concentration of substance is not within the detection range exactly, an extra dilution or concentration should be taken for the sample.
5. It is recommended to take a pre-test if your sample is not listed in the instruction book.
6. The experimental results are closely related to the situation of reagents, operations, environment and so on. Novus Biologicals will guarantee the quality of the kits only, and NOT be responsible for the sample consumption caused by using the assay kits. It is better to calculate the possible usage of sample and reserve sufficient samples before use.