# GSI Total Antioxidant Capacity Assay DataSheet

**Product name**: Nori® Total Antioxidant Capacity Assay
**Number of tests**: 200
**Sample type**: Serum, plasma, urine, tissue extracts, cell lysates, cell culture media, other biological fluids

**Product overview**:

An antioxidant is a molecule that inhibits the oxidation of other molecules. Oxidation is a chemical reaction that transfers electrons or hydrogen from a substance to an oxidizing agent. Oxidation reactions can produce free radicals. In turn, these radicals can start chain reactions. When the chain reaction occurs in a cell, it can cause damage or death to the cell. Antioxidants terminate these chain reactions by removing free radical intermediates, and inhibit other oxidation reactions. They do this by being oxidized themselves, so antioxidants are often reducing agents such as thiols, ascorbic acid, or polyphenols.[1] Although oxidation reactions are crucial for life, they can also be damaging; plants and animals maintain complex systems of multiple types of antioxidants, such as glutathione, vitamin C, vitamin A, and vitamin E as well as enzymes such as catalase, superoxide dismutase and various peroxidases. Insufficient levels of antioxidants, or inhibition of the antioxidant enzymes, cause oxidative stress and may damage or kill cells. Oxidative stress is damage to cell structure and cell function by overly reactive oxygen-containing molecules and chronic excessive inflammation. Oxidative stress seems to play a significant role in many human diseases, including cancers. Oxidative stress can be considered to be both the cause and the consequence of some diseases.

Antioxidants are classified into two broad divisions, depending on whether they are soluble in water (hydrophilic) or in lipids (lipophilic). In general, water-soluble antioxidants react with oxidants in the cell cytosol and the blood plasma, while lipid-soluble antioxidants protect cell membranes from lipid peroxidation.[1] These compounds may be synthesized in the body or obtained from the diet.[2] The different antioxidants are present at a wide range of concentrations in body fluids and tissues, with some such as glutathione or ubiquinone mostly present within cells, while others such as uric acid are more evenly distributed. Some antioxidants are only found in a few organisms and these compounds can be important in pathogens and can be virulence factors.[3] The amount of protection provided by any one antioxidant will also depend on its concentration, its reactivity towards the particular reactive oxygen species being considered, and the status of the antioxidants with which it interacts.[2] Some compounds contribute to antioxidant defense by chelating transition metals and preventing them from catalyzing the production of free radicals in the cell. Particularly important is the ability to sequester iron, which is the function of iron-binding proteins such as transferrin and ferritin.[4]

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**Principle of the assay:**

Measurement of total antioxidant capacity (TAC) in body fluids, tissues and cells, may have important prognostic and diagnostic value.[5] This kit is for quantification of TAC in various specimens. Similar to other kits, The principle of the antioxidant assay is formation of a TMB (3,3',5,5'-tetramethylbenzidine) radical in the presence of HRP (horseradish peroxidase) and hydrogen peroxide, which oxidizes the TMB to produce a radical cation, TMB diimine, a soluble chromogen that is blue in color and can be determined spectrophotometrically at 450 nm. Antioxidants suppress the production of the radical cation in a concentration dependent manner and the color intensity decreases proportionally. Trolox, a water-soluble vitamin E analog, serves as a standard for quantification. This kit provides all of the reagents required for an efficient measurement of the TAC.

## References


1. Sies, Helmut (1997). *Experimental physiology* 82 (2): 291–5.
2. Vertuani, (2004). *Current Pharmaceutical Design* 10 (14): 1677–94.
3. Miller, RA; Britigan, BE (1997). *Clinical Microbiology Reviews* 10 (1): 1–18.
4. Imlay, James A. (2003). *Annual Review of Microbiology* 57: 395–418.
5. Rice-Evans CA (2000) Free Radic Res 33, S59-66,


**Storage**

The kit is shipped on wet ice and should be stored at 2-8°C.

This package insert must be read in its entirety before using this product.

## Materials provided

| Description | Quantity | Description | Quantity | Description | Quantity |
|---|---|---|---|---|---|
| Trolox Standard | 4 | 20 x Assay Buffer | 1 | DataSheet | 1 |
| HRP | 1 | 20 x Diluent | 1 | 96-well plate sheet | 1 |
| TMB Substrate | 1 | Stop Solution | 1 | MSDS/CoA | 1 |

Bring all reagents to room temperature before use.

**HRP (horseradish peroxidase)** (20 µL) – The vial contains peroxidase sufficient for two 96-well plates and can be stored at 4 °C for up to 6 months if not used immediately. Make 1:600 dilution in 1 x Assay Buffer. Centrifuge for 1 min at 6000 x g to bring down the material prior to open the tube. If the volume is less than 20 µl, add 1 x Diluent to reach the total volume of 20 µl, vortex 30 sec and allow it to sit for 5 min prior to use. If the entire 96-well plate is used, add 8.5 µL of HRP to 5 mL of 1 x Assay Buffer to make working dilution of HRP and vortex 30 sec.

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**TMB Substrate** (4 mL) –The vial contains sufficient TMB Substrate for two 96-well plates. Make 1:3 dilution in 1 x Assay Buffer. If the entire 96-well plate is used, add 1.7 mL of TMB to 3.4 mL of 1 x Assay Buffer to make working dilution of TMB Substrate and vortex 30 sec prior to the assay. The rest of undiluted TMB Substrate can be stored at 4°C for up to 6 months. DO NOT FREEZE.

**Trolox Standard** (4 vials) – The lyophilized Trolox Standard should be stored at 4°C for up to 6 months, if not used immediately. Centrifuge for 1 min at 6000 x g to bring down the material prior to open the vial. Each vial contains the standard sufficient for a 96-well plate. Add 100 µL of 1 x Assay Buffer to a vial to make a high concentration of 400 µM, vortex 30 sec, label as tube #1; prepare the rest of the standards as Table 1.

Table 1 Trolox Standard Preparation

| Tubes | 400 µM Trolox (µL) | 1 x Assay Buffer (µL) | Resulting Trolox Concentration (µM) |
|---|---|---|---|
| 1 | 100 | 0 | 400 |
| 2 | 50 of tube #1 | 50 | 200 |
| 3 | 50 of tube #2 | 50 | 100 |
| 4 | 50 of tube #3 | 50 | 50 |
| 5 | 50 of tube #4 | 50 | 25 |
| 6 | 0 | 50 | 0 |

**20 x Assay Buffer**, 20 mL- Dilute to 1 x Assay Buffer with 1 x Diluent and mix well prior to use.
**20 x Diluent**, 20 mL- Dilute to 1 x Diluent with deionized distilled water prior to use.
**Stop Solution**, 10.5 mL.

### Materials required but not provided

96-well microplate, flat bottom

### Assay Procedure

1. Vortex briefly the standards and samples prior to the assay. Add 20 µL of the Standard or sample per well and use duplicate wells for each of standards and samples.
2. Add 50 µL of the working dilution of the HRP to each well.
3. Add 50 µL of the working dilution of the TMB Substrate Solution to each well. Gently tap the plate to ensure thorough mixing. Incubate for up to 20 minutes at room temperature. Avoid placing the plate in direct light.
4. Add 50 µL of Stop Solution to each well. Gently tap the plate to ensure thorough mixing.
5. Determine the optical density of each well immediately, using a microplate reader set to 450 nm.

## Precaution and Technical Notes

1. A standard curve should be generated for each set of samples assayed. Thorough mixing of the standard at each of dilution step is essential to generate a normal calibration curve for quantification of unknown sample.
2. EDTA can interfere with the TAC assay and should not be present in any sample including plasma sample.
3. Antioxidant levels of the Test Samples should fall within the range of the standard curve. Samples containing antioxidant levels between 25-400 µM (Trolox equivalents) can be tested without dilution or concentration. If dilution is needed, the test samples should be diluted with 1 x Assay Buffer and vortex 30 sec prior to assay to bring the antioxidant level with range. The dilution factors must be used to calculate the concentration.
4. The Stop Solution is an acid solution, handle with caution.
5. This kit should not be used beyond the expiration date on the label.
6. Use a fresh reagent reservoir and pipette tips for each step.
7. It is recommended that all standards and samples be assayed in duplicate.
8. Avoid microbial contamination of reagents and buffers. This may interfere with the sensitivity of the assay.

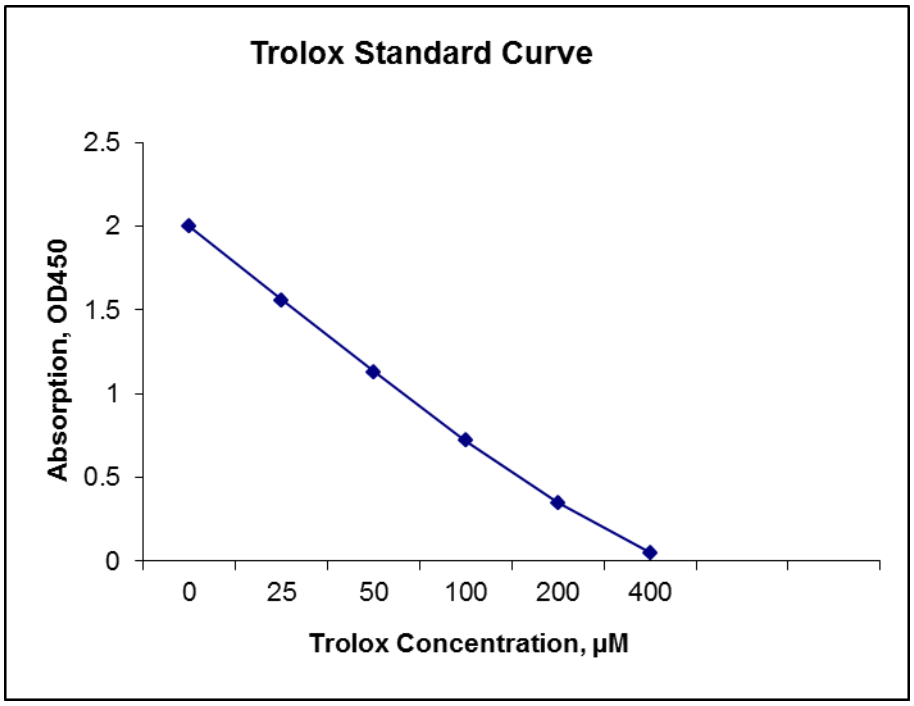
## Calculation of Results

1. Average the duplicate readings for each standard, control, and sample.
2. Create a standard curve by plotting the average absorbance of each Trolox Standard as a function of the final Trolox concentration (µM).
3. Calculate the antioxidant concentration of the Test Sample using the equation obtained from the linear regression of the standard curve (see Figure 1 for standard curve example).

X (µM) = [y($A_{450}$)-intercept)/slope] x dilution factor

X (µM) –Antioxidant concentration (M) [relative to the concentration of the Trolox standard]
Y($A_{450}$)- The average absorbance of the Test Sample at 450 nm.
Intercept- Intercept of the Y axis by the standard curve.
Slope- Slope of the standard curve, a negative value.
Dilution factor-Fold dilution of the original sample (will be used only if sample was diluted prior to adding to the well).

Figure 1 Example of Trolox Standard Curve




**Detection Range**

25-400 µM

**Assay Sensitivity**

25 µM

**Assay Precision**

Intra-Assay %CV: 7; Inter-Assay %CV: 9

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