# TECHNICAL MANUAL

# Sorbitol Dehydrogenase (SORD) Activity Assay Kit

- **SKU CODE:** BR00011
- **SIZE:** 100 wells
- **DETECTION PRINCIPLE:** Colorimetric
- **RUO:** Research-Use-Only

Revised: 10/26

# Sorbitol Dehydrogenase (SORD) Activity Assay Kit

*Please read entire manual carefully before starting experiment.*

## Table of Contents

**1. Key Features** ..... 3

**2. Storage & Expiry** ..... 3

**3. Product Description** ..... 3

**4. Kit Contents** ..... 4

**5. Important Notes** ..... 4

**6. Reagent Preparation** ..... 5

**7. Sample Preparation** ..... 6

**8. Assay Procedure** ..... 6

**9. Data Analysis** ..... 7

# 1. Key Features

**Specification:**

100 wells

**Measuring instrument:**

Microplate reader ($O.D._{492\ nm}$)

**Sample type:**

Crude cell/tissue lysates

# 2. Storage & Expiry

This product should be stored according to the conditions listed for each kit component. Kit components are stable for several years if stored and handled properly. For detailed storage instructions of individual kit components, please refer to Section 4. The expiration date is indicated on the outer label of the kit box.

# 3. Product Description

The SORD enzyme activity assay is based on reduction of the tetrazolium salt INT in a NADH-coupled enzymatic reaction to formazan, which exhibits an absorption maximum at 492 nm ($\varepsilon$ = 18 $mM^{-1}cm^{-1}$) and allows for measurement of SORD activity present in crude cell/tissue lysates.

# 4. Kit Contents

| No | Component Name | Size | Storage |
|---|---|---|---|
| 1 | SORD Assay Solution | 5 ml (for 100 wells) | -80°C (shield solution from light during assay) |
| 2 | 10x SORD Substrate | 0.5 ml | -80°C |
| 3 | 10x Sample Buffer | 25 ml | 4°C |

***Note:*** *All reagents must be stored according to the specified conditions listed in the table above. Do not mix reagents from different kits, as this may compromise assay performance. For reagents provided in small volumes, centrifuge briefly before use to ensure complete recovery of the contents.*

### Additional materials required:

1. 37°C incubator (not a $CO_2$ incubator).
2. Microplate Reader (492 nm).
3. Plain (uncoated) 96-well plate.
4. Refrigerated microcentrifuge (~14,000 rpm) and tissue homogenizer.
5. Precision pipettes and disposable pipette tips. Distilled water.
6. Protein assay reagents.

# 5. Important Notes

1. This assay kit is intended for Research Use Only. Assay Genie assumes no responsibility for any issues or legal liabilities arising from the use of this kit for clinical diagnostics or any other unauthorized purposes.
2. Please read the instructions carefully before beginning the assay. Ensure that all instruments are correctly calibrated. Strict adherence to the protocol is essential for accurate results.
3. Appropriate laboratory safety precautions must be followed, including the use of lab coats and latex gloves.

4. If the concentration of the target substance falls outside the detection range, please adjust the sample by performing further dilution or concentration as needed.
5. If your sample type is not listed in the instruction manual, we strongly recommend performing a preliminary test to confirm compatibility.
6. Experimental outcomes depend on multiple factors including reagent integrity, handling technique, and laboratory conditions. While Assay Genie guarantees the quality of our kits, we are not responsible for any loss of samples during use. We advise calculating sample requirements in advance and ensuring adequate sample volume is reserved before starting the assay.
7. The assay solution contains dimethyl sulfoxide (DMSO) and iodonitrotetrazolium (INT). Please refer to the product webpage or contact Assay Genie for material SDS information.

# 6. Reagent Preparation

1. **1x Sample Buffer:** Prepare 1x Sample Buffer by diluting 10x Sample Buffer with $dH_2O$.
2. **Reagent thawing:** Keep thawed SORD Assay Solution and 10x SORD Substrate on ice. Do not over thaw. Freeze solutions immediately after use.
3. **Control solution:** Prepared by mixing 1 part of $dH_2O$ and 10 parts of SORD Assay Solution, e.g. 50 µl $dH_2O$ mixed with 500 µl SORD Assay Solution.
4. **Reaction solution:** Prepared by mixing 1 part of 10x SORD Substrate and 10 parts of SORD Assay Solution, e.g. 50 µl 10x SORD Substrate mixed with 500 µl SORD Assay Solution.

| Item | Control solution | Reaction solution |
|---|---|---|
| $dH_2O$ | 50 µl | – |
| 10x SORD Substrate | – | 50 µl |
| SORD Assay Solution | 500 µl | 500 µl |

# 7. Sample Preparation

## Cell/tissue extracts

1. PBS-washed cell/tissue samples should be frozen at -80°C prior to homogenization. At least ~$10^5$ harvested cells or 2 mg of tissue should be used for lysate preparation. Freeze thawing, mechanical grinding, or sonication facilitates protein extraction.
2. Bring up freeze-thawed cells in 50–100 µl ice-cold 1x Sample Buffer by pipetting up and down. Adherent cells after deep freezing can be directly harvested from plate by scraping. Leave lysate on ice for 5 min with agitation. Freeze-thawed tissue is mechanically homogenized in ice-cold 1x Sample Buffer (~5 mg tissue in 500 µl).
3. Centrifuge lysate in a cold microfuge at ~14,000 rpm for 5 min. Supernatant is harvested and stored at -80°C.
4. Perform protein assay to determine lysate (supernatant) protein concentration. A suggested sample protein concentration range is 0.2 – 3 mg/ml. Normalization of sample protein concentration is recommended.

# 8. Assay Procedure

1. **Add samples:** Use a plain (uncoated) 96-well plate for the assay. Add 20 µl of each sample to duplicate wells (for control set and reaction set).
2. **Add solutions and incubate:** Add 50 µl control solution to one set of sample wells and 50 µl reaction solution to the other set of sample wells. Mix contents by gentle agitation for 10 sec. Cover plate and incubate in a 37°C incubator for 30 min or 60 min. Do not use $CO_2$ incubator. Cherry red color should gradually appear in sample wells.
3. **Measure:** Measure $O.D._{492\,nm}$ using a plate reader. Subtract control well reading from reaction well reading for each sample. Use the subtracted reading (ΔO.D.) for enzyme activity calculation.

# 9. Data Analysis

**Calculation:**

If incubation for 30 min, SORD enzyme activity in IU/L = μmol/(L•min) = ΔO.D. × 1000 × 70 μl/(30 min × 0.4 cm × 18 × 20 μl) = **ΔO.D. × 16.2**

If incubation for 60 min, SORD activity = **ΔO.D. × 8.1**

Enzyme activity may be presented as units/g proteins or normalized by GAPDH enzyme activity. Increase sample protein concentration if detected enzyme activity is too low.

***Note:***

***ΔO.D.:*** *reaction well reading minus control well reading at 492 nm.*

***70 μl:*** *total volume per well (20 μl sample + 50 μl control or reaction solution).*

***min:*** *incubation time.*

***0.4 cm:*** *light path length.*

***18:*** *extinction coefficient of formazan at 492 nm ($mM^{-1}cm^{-1}$).*

***20 μl:*** *sample volume.*

***1000:*** *conversion factor from mmol to μmol.*

**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.