Product Information

α-Ketoglutarate Dehydrogenase Activity Colorimetric Assay Kit

Catalog Number MAK189
Storage Temperature –20 °C

TECHNICAL BULLETIN

Product Description
α-Ketoglutarate Dehydrogenase (α-KGDH, EC 1.2.4.2) forms the E1 subunit of the mitochondrial 2-oxoglutarate dehydrogenase complex that catalyzes the conversion of 2-oxoglutarate to succinyl-CoA and CO₂ during the Krebs cycle. Decreased α-KGDH complex activity has been associated with oxidative stress and neurodegenerative disorders such as Alzheimer’s and Parkinson’s diseases.¹,²

The α-Ketoglutarate Dehydrogenase Activity Colorimetric Assay Kit provides a simple procedure for measuring α-ketoglutarate dehydrogenase activity in a variety of tissues, cells, and isolated mitochondria. α-KGDH activity is determined by measuring a colorimetric product with absorbance at 450 nm (A₄₅₀) proportional to the enzymatic activity present. One unit of α-ketoglutarate dehydrogenase is the amount of enzyme that generates 1.0 µmole of NADH per minute at pH 7.5 at 37 °C.

Components
The kit is sufficient for 100 assays in 96 well plates.

- KGDH Assay Buffer
  - Catalog Number MAK189A
  - 25 mL

- KGDH Substrate
  - Catalog Number MAK189B
  - 1 vl

- KGDH Developer
  - Catalog Number MAK189C
  - 1 vl

- NADH Standard
  - Catalog Number MAK189D
  - 1 vl

- KGDH Positive Control
  - Catalog Number MAK189E
  - 50 µL

Reagents and Equipment Required but Not Provided
- 96 well flat-bottom plate – It is recommended to use clear plates for colorimetric assays.
- Spectrophotometric multiwell plate reader
- Saturated ammonium sulfate (∼4.1 M, optional for samples containing small interfering molecules)
- Mitochondria Isolation Kit (optional for mitochondria samples, Catalog Number MITOISO1 for tissue, MITOISO2 for cells, MITOISO3 for yeast, or equivalent)

Precautions and Disclaimer
This product is for R&D use only, not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions
Briefly centrifuge vials before opening. Use ultrapure water for the preparation of reagents. To maintain reagent integrity, avoid repeated freeze/thaw cycles.

- KGDH Assay Buffer – Store at –20 °C or 2–8 °C. Allow buffer to come to room temperature before use.
- KGDH Substrate – Reconstitute with 220 µL of water. Mix well by pipetting. Aliquot and store at –20 °C. Keep on ice during use. Use within 2 months.
- KGDH Developer – Reconstitute with 220 µL of water. Mix well by pipetting. Aliquot and store at –20 °C. Use within 2 months.
NADH Standard – Reconstitute with 400 µL of water to generate a 1.25 mM (1.25 nmole/µL) NADH Standard Solution. Aliquot and store at −20 °C. Keep on ice during use. Use within 2 months.

KGDH Positive Control – Reconstitute with 100 µL of KGDH Assay Buffer. Mix well by pipetting. Aliquot and store at −20 °C. Keep on ice during use. Use within 2 months.

Storage/Stability
The kit is shipped on wet ice and storage at −20 °C, protected from light, is recommended.

Procedure
All samples and standards should be run in duplicate.

NADH Standards for Colorimetric Detection
Add 0, 2, 4, 6, 8, and 10 µL of the 1.25 mM NADH Standard Solution into a 96 well plate, generating 0 (blank), 2.5, 5.0, 7.5, 10, and 12.5 nmole/well standards. Add KGDH Assay Buffer to each well to bring the volume to 50 µL.

Sample Preparation
Tissue samples (10 mg) or cells (1 × 10⁶) can be homogenized in 100 µL of ice cold KGDH Assay Buffer. Keep on ice for 10 minutes. Centrifuge the samples at 10,000 × g for 5 minutes to remove insoluble material. Transfer supernatant to a fresh tube.

When analyzing KGDH activity in mitochondria, it is recommended to isolate the mitochondria from fresh tissue or cells.

Add 5–50 µL of the samples into duplicate wells. Bring samples to a final volume of 50 µL using KGDH Assay Buffer.

Notes: For unknown samples, it is suggested to test several sample dilutions to ensure the readings are within the linear range of the standard curve.

Small molecules in some tissues such as liver may interfere with the assay. To remove small molecules, it is suggested to use an ammonium sulfate precipitation method. Pipette 50–100 mL of lysate into a fresh tube, add 2× volume of saturated ammonium sulfate (−4.1 M at room temperature) and keep on ice for 20 minutes. Centrifuge at 10,000 × g for 5 minutes, remove and discard the supernatant, and resuspend the pellet to the original volume with KGDH Assay Buffer.

For samples exhibiting significant background, include a Sample Blank for each sample by omitting the KGDH Substrate. The Sample Blank readings can then be subtracted from the sample readings.

For a positive control (optional), add 2–10 µL of the KGDH Positive Control solution to the desired wells. Adjust the final volume to 50 µL with the KGDH Assay Buffer.

Assay Reaction
1. Set up Reaction Mixes according to the scheme in Table 1. 50 µL of Reaction Mix is required for each reaction (well).

Table 1. Reaction Mixes

<table>
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<th>Reagent</th>
<th>Standards, Controls, and Samples</th>
<th>Sample Blank</th>
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<tr>
<td>KGDH Assay Buffer</td>
<td>46 µL</td>
<td>48 µL</td>
</tr>
<tr>
<td>KGDH Developer</td>
<td>2 µL</td>
<td>2 µL</td>
</tr>
<tr>
<td>KGDH Substrate</td>
<td>2 µL</td>
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2. Add 50 µL of the appropriate Reaction Mix to each of the wells. Mix well using a horizontal shaker or by pipetting.

3. Measure the absorbance (A₄₅₀) in a microplate reader in kinetic mode for 10–60 minutes at 37 °C. Protect the plate from light during the incubation. It is recommended to take absorbance readings every minute. Note: Incubation time depends on the activity of KGDH in the samples.

4. Continue taking measurements until the value of the most active sample is greater than the value of the highest standard (12.5 nmole/well). At this time the most active sample is near or exceeds the end of the linear range of the standard curve.

Note: The NADH Standards can be read at the end of the incubation time.
Results
Calculations
Plot the absorbance ($A_{450}$) for each well versus time.

Choose two time points (T1 and T2) in the linear range of the plot and determine the $A_{450}$ at each time (ABS1 and ABS2).

Note: It is essential that ABS1 and ABS2 fall within the linear range of the standard curve.

Correct for the background by subtracting the measurement obtained for the 0 (blank) NADH standard from that of the standards, controls, and samples. Background values can be significant and must be subtracted from all readings. Use the values obtained from the appropriate NADH Standards to plot a standard curve.

Note: A new standard curve must be set up each time the assay is run.

Calculate the change in absorbance from T1 to T2 for the samples.

$$\Delta \text{ABS} = \text{ABS2} - \text{ABS1}$$

Subtract the Sample Blank $\Delta \text{ABS}$ value from the Sample $\Delta \text{ABS}$ reading to obtain the corrected measurement. Using the corrected measurement, determine the amount of NADH (nmole/well) generated by the KGDH assay between T1 and T2 ($S_a$).

$\alpha$-Ketoglutarate Dehydrogenase (KGDH) activity

KGDH Activity = \(\frac{S_a}{(\text{Reaction Time}) \times S_v}\)

where:

- $S_a$ = Amount of NADH (nmole) generated in unknown sample well between T1 and T2 from standard curve
- Reaction Time = T2 – T1 (minutes)
- $S_v$ = sample volume (mL) added to well

KGDH activity is reported as

n mole/min/mL = milliunit/mL.

One unit of $\alpha$-ketoglutarate dehydrogenase is the amount of enzyme that generates 1.0 µmole of NADH per minute at pH 7.5 at 37 °C.

Sample Calculation:

Amount of NADH ($S_a$) = 5.84 nmole (from standard curve)

(T1) = 3 minutes
(T2) = 32 minutes

Sample volume ($S_v$) = 0.050 mL

KGDH activity in sample well:

n mole/min/mL = \(\frac{5.84 \text{ nmole/well}}{(32 \text{ min} - 3 \text{ min}) \times 0.050 \text{ mL/well}}\) = 4.03 (milliunits/mL)

References
## Troubleshooting Guide

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<td>Allowing the reagents to sit for extended times on ice</td>
<td>Prepare fresh Reaction Mixes before each use</td>
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<td>Concentrate or dilute samples so readings are in the linear range</td>
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