

# Product Information

## Oxaloacetate Assay Kit

Catalog Number **MAK070**

Storage Temperature –20 °C

## TECHNICAL BULLETIN

### Product Description

Oxaloacetate (OAA) is an intermediate of both the TCA cycle and gluconeogenesis. It precedes citrate, which is formed by the citrate synthase-mediated condensation of acetyl-CoA and OAA. OAA is also formed by the deamidation of aspartate or condensation of CO<sub>2</sub> with pyruvate or PEP. Since mammals do not possess the enzymatic machinery to form TCA cycle intermediates from acetyl-CoA, OAA is one of the anaplerotic entry points via pyruvate and pyruvate carboxykinase.

This kit provides a simple, sensitive and rapid means of quantifying OAA in a variety of samples. In this kit, oxaloacetate concentration is determined by a coupled enzyme assay, which results in a colorimetric (570 nm)/fluorometric ( $\lambda_{\text{ex}} = 535/\lambda_{\text{em}} = 587$  nm) product, proportional to the oxaloacetate present. Typical detection ranges for this kit are 2–10 nmole (colorimetric) and 0.2–1 nmole (fluorometric).

### Components

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

Oxaloacetate Assay Buffer Catalog Number MAK070A	25 mL
Oxaloacetate Probe, in DMSO Catalog Number MAK070B	0.2 mL
Oxaloacetate Enzyme Mix Catalog Number MAK070C	1 vL
Developer Catalog Number MAK070D	1 vL
Oxaloacetate Standard, 10 µmole Catalog Number MAK070E	1 vL

### Reagents and Equipment Required but Not Provided.

- 96 well flat-bottom plate – It is recommended to use black plates with clear bottoms for fluorescence assays and clear plates for colorimetric assays.
- Fluorescence or spectrophotometric multiwell plate reader
- 10 kDa Molecular Weight Cut-Off (MWCO) Spin Filter

### Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material 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.

OAA Assay Buffer – Allow buffer to come to room temperature before use.

OAA Probe – Warm to room temperature before use to melt frozen DMSO. Aliquot and store protected from light and moisture at –20 °C. Upon thawing, the OAA Probe is ready-to-use in the colorimetric assay.

For the fluorescence assay, dilute an aliquot of the colorimetric OAA Probe Solution 5 to 10-fold with OAA Assay Buffer, just prior to use. This will reduce the background of the fluorescence assay.

OAA Enzyme Mix and Developer – Reconstitute each vial with 220 µL of OAA Assay Buffer. Mix well by pipetting, then aliquot and store at –20 °C. Use within 2 months of reconstitution.

OAA Standard – Reconstitute in 100  $\mu$ L of water to generate a 100 mM (100 nmole/ $\mu$ L) OAA Standard Solution. Mix well by pipetting, then aliquot and store at –20 °C.

#### 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.

#### Oxaloacetate Standards for Colorimetric Detection

Dilute 10  $\mu$ L of the 100 mM (100 nmole/ $\mu$ L) OAA Standard Solution with 990  $\mu$ L of water to generate a 1 mM (1 nmole/ $\mu$ L) standard solution. Add 0, 2, 4, 6, 8, and 10  $\mu$ L of the 1 mM OAA standard into a 96 well plate, generating 0 (blank), 2, 4, 6, 8, and 10 nmole/well standards. Add OAA Assay Buffer to each well to bring the volume to 50  $\mu$ L.

#### Oxaloacetate Standards for Fluorometric Detection

Prepare a 1 mM standard solution as for the colorimetric assay. Take 10  $\mu$ L of the 1 mM standard and add to 90  $\mu$ L of water to generate a 0.1 mM standard solution. Add 0, 2, 4, 6, 8, and 10  $\mu$ L of the 0.1 mM OAA standard solution into a 96 well plate, generating 0 (blank), 0.2, 0.4, 0.6, 0.8, and 1.0 nmole/well standards. Add OAA Assay Buffer to each well to bring the volume to 50  $\mu$ L.

#### Sample Preparation

Both the colorimetric and fluorometric assays require 50  $\mu$ L of sample for each reaction (well).

Tissue (20 mg) or cells ( $2 \times 10^6$ ) should be rapidly homogenized with 100  $\mu$ L of OAA Assay Buffer. Centrifuge at 15,000  $\times g$  for 10 minutes to remove insoluble materials. Bring samples to a final volume of 50  $\mu$ L with OAA Assay Buffer.

**Note:** Because enzymes in samples may interfere with the assay, samples should be deproteinized with a 10 kDa MWCO spin filter prior to addition to the reaction.

For unknown samples, it is suggested to test several sample volumes to make sure the readings are within the standard curve range.

**Note:** Pyruvate in samples can generate background for the assay. To remove the pyruvate background, include a blank sample for each sample by omitting the OAA Enzyme Mix in the Reaction Mix.

#### Assay Reaction

1. Set up the Reaction Mixes according to the scheme in Table 1. 50  $\mu$ L of the appropriate Reaction Mix is required for each reaction (well).

**Table 1.**  
Reaction Mixes

Reagent	Blank Sample	Samples and Standards
OAA Assay Buffer	46 $\mu$ L	44 $\mu$ L
OAA Enzyme Mix	–	2 $\mu$ L
OAA Developer	2 $\mu$ L	2 $\mu$ L
OAA Probe	2 $\mu$ L	2 $\mu$ L

2. Add 50  $\mu$ L of the appropriate Reaction Mix to each of the blank, standard, and test wells. Mix well using a horizontal shaker or by pipetting, and incubate at room temperature for 30 minutes, protected from light.
3. For colorimetric assays, measure the absorbance at 570 nm ( $A_{570}$ ). For fluorometric assays, measure fluorescence intensity ( $\lambda_{ex} = 535/\lambda_{em} = 587$  nm).

## Results

### Calculations

The background for the assays is the value obtained for the 0 (blank) OAA standard. Correct for the background by subtracting the blank value from all readings.

Background values can be significant and must be subtracted from all readings. Use the values obtained from the appropriate OAA standards to plot a standard curve.

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

Subtract the blank sample value from the sample reading to obtain the corrected measurement. Using the corrected measurement, the amount of OAA present in the sample may be determined from the standard curve.

### Concentration of OAA

$$S_a/S_v = C$$

$S_a$  = Amount of OAA in unknown sample (nmole) from standard curve

$S_v$  = Sample volume ( $\mu$ L) added into the wells

$C$  = Concentration of OAA in sample

Oxaloacetate molecular weight: 132.07 g/mole.

### Sample Calculation

Oxaloacetate amount ( $S_a$ ) = 8.07 nmole

Sample volume ( $S_v$ ) = 50  $\mu$ L

Concentration of OAA in sample

$$8.07 \text{ nmole}/50 \mu\text{L} = 0.1614 \text{ nmole}/\mu\text{L}$$

$$0.1614 \text{ nmole}/\mu\text{L} \times 132.07 \text{ ng/nmole} = 21.32 \text{ ng}/\mu\text{L}$$

**Troubleshooting Guide**

<b>Problem</b>	<b>Possible Cause</b>	<b>Suggested Solution</b>
Assay not Working	Assay Buffer Ice Cold	Assay Buffer must be at room temperature
	Omission of step in procedure	Refer and follow Technical Bulletin precisely
	Plate reader at incorrect wavelength	Check filter settings of instrument
	Type of 96 well plate used	For fluorescence assays, use black plates with clear bottoms. For colorimetric assays, use clear plates
Samples with erratic readings	Samples prepared in different buffer	Use the Assay Buffer provided or refer to Technical Bulletin for instructions
	Samples were not deproteinized	Use a 10 kDa MWCO spin filter to deproteinize samples
	Cell/Tissue culture samples were incompletely homogenized	Repeat the sample homogenization, increasing the length and extent of homogenization step.
	Samples used after multiple freeze-thaw cycles	Aliquot and freeze samples if samples will be used multiple times
	Presence of interfering substance in the sample	If possible, dilute sample further
	Use of old or inappropriately stored samples	Use fresh samples and store correctly until use
Lower/higher readings in samples and standards	Improperly thawed components	Thaw all components completely and mix gently before use
	Use of expired kit or improperly stored reagents	Check the expiration date and store the components appropriately
	Allowing the reagents to sit for extended times on ice	Prepare fresh Reaction Mix before each use
	Incorrect incubation times or temperatures	Refer to Technical Bulletin and verify correct incubation times and temperatures
	Incorrect volumes used	Use calibrated pipettes and aliquot correctly
Non-linear standard curve	Use of partially thawed components	Thaw and resuspend all components before preparing the reaction mix
	Pipetting errors in preparation of standards	Avoid pipetting small volumes
	Pipetting errors in the Reaction Mix	Prepare a Reaction Mix whenever possible
	Air bubbles formed in well	Pipette gently against the wall of the plate well
	Standard stock is at incorrect concentration	Refer to the standard dilution instructions in the Technical Bulletin
	Calculation errors	Recheck calculations after referring to Technical Bulletin
	Substituting reagents from older kits/lots	Use fresh components from the same kit
Unanticipated results	Samples measured at incorrect wavelength	Check the equipment and filter settings
	Samples contain interfering substances	If possible, dilute sample further
	Sample readings above/below the linear range	Concentrate or dilute samples so readings are in the linear range

MF,LS,MAM 03/12-1