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Product Information

Citrate Synthase Activity Assay Kit

Catalog Number **MAK193**
Storage Temperature –20 °C

TECHNICAL BULLETIN

Product Description

Citrate Synthase (CS; EC 2.3.3.1) is a mitochondrial enzyme found in all cells capable of oxidative metabolism. It catalyzes the synthesis of citrate from oxaloacetate in the Krebs tricarboxylic acid cycle.[1,2] CS is involved in energy production, lipogenesis, and cholesterologenesis, and its activity follows a circadian pattern.[3]

The Citrate Synthase Activity Assay kit provides a simple and direct procedure for measuring the activity of CS in a variety of tissues, cell cultures (adherent or suspension), and purified mitochondria. CS activity is determined using a coupled enzyme reaction, which results in a colorimetric (412 nm) product proportional to the enzymatic activity present. One unit of CS is the amount of enzyme that generates 1.0 μmole CoA per minute at 25 °C and pH 7.2.

Components

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

CS Assay Buffer Catalog Number MAK193A	25 mL
CS Substrate Mix Catalog Number MAK193B	1 vl
CS Developer Catalog Number MAK193C	1 vl
GSH Standard, Reduced Catalog Number MAK193D	1 vl
CS Positive Control Catalog Number MAK193E	1 vl

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
- 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.

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

CS Substrate Mix – Reconstitute with 220 μL of water. Mix well by pipetting, then aliquot and store at –20 °C. Place on ice during use. Use within 2 months of reconstitution.

CS Developer – Reconstitute with 1 mL of CS Assay Buffer. Mix well by pipetting, then aliquot and store at –20 °C. Keep on ice during use. Use within 2 months of reconstitution.

GSH Standard – Reconstitute with 100 μL of water to prepare 20 mM GSH Standard Solution. Mix well by pipetting, then aliquot and store at –20 °C. Keep on ice during use. Use within 2 months of reconstitution.

CS Positive Control – Reconstitute with 100 µL of CS Assay Buffer. Mix well by pipetting, then aliquot and store at –20 °C. Keep on ice during use. Use within 2 months of reconstitution.

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.

GSH Standard Curve Preparation
Dilute 10 µL of the 20 mM GSH Standard Solution with 90 µL of the CS Assay Buffer to prepare a 2 mM (2 nmole/µL) GSH Standard Solution. Add 0, 4, 8, 12, 16, and 20 µL of the 2 mM GSH Standard Solution into a 96 well plate to generate 0 (blank), 8, 16, 24, 32, and 40 nmole/well standards. Add CS Assay Buffer to each well to bring the volume to 50 µL.

Sample Preparation
Tissue samples (10 mg) or cells (1×10^6) can be homogenized in 100 µL of ice cold CS 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 CS activity in mitochondria, it is recommended to isolate the mitochondria from fresh tissue or cells.

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

Add 1–50 µL of the samples into duplicate wells. Bring samples to a final volume of 50 µL using CS Assay Buffer.

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

For a positive control (optional), dilute 10 µL of the CS Positive Control in 990 µL of CS Assay Buffer. Add 2–20 µL of diluted CS Positive Control in the desired wells. Adjust final volume to 50 µL using CS Assay Buffer.

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

Table 1.
Reaction Mixes

Reagent	Standards and Samples	Sample Blank
CS Assay Buffer	43 µL	45 µL
CS Developer	5 µL	5 µL
CS Substrate Mix	2 µL	–

2. Add 50 µL of the appropriate Reaction Mix to each of the wells. Mix well using a horizontal shaker or by pipetting.

3. Incubate the plate at 25 °C. Take the initial measurement. Measure the absorbance at 412 nm [$(A_{412})_{initial}$] at the initial time ($T_{initial}$).

4. Continue to incubate the plate at 25 °C taking measurements (A_{412}) every 5 minutes for 20–40 minutes.

   Note: Incubation time depends on the activity of CS in the samples.

5. Continue taking measurements until the value of the most active sample is greater than the value of the highest standard (40 nmole/well). At this time the most active sample is near or exceeds the end of the linear range of the standard curve.

6. The final absorbance measurement [$(A_{412})_{final}$] for calculating the enzyme activity would be the penultimate reading or the value before the most active sample is near or exceeds the end of the linear range of the standard curve (see step 5). The time of the penultimate reading is T_{final}.

   Note: It is essential that $(A_{412})_{final}$ falls within the linear range of the standard curve.

Results

Calculations

Correct for the background by subtracting the final measurement [$(A_{412})_{final}$] obtained for the 0 (blank) GSH Standard from the final measurement [$(A_{412})_{final}$] of the standards and samples. Background values can be significant and must be subtracted from all readings. Use the values obtained from the appropriate GSH 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 measurement from $T_{initial}$ to T_{final} for the samples.

$$\Delta A_{412} = (A_{412})_{final} - (A_{412})_{initial}$$

Subtract the Sample Blank ΔA_{412} value from the sample ΔA_{412} reading to obtain the corrected measurement. Using the corrected measurement, determine the amount of GSH (nmole/well) generated by the CS assay between $T_{initial}$ and T_{final} (S_a).

CS activity:

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

where:

S_a = Amount of GSH (nmole) generated in unknown sample well between $T_{initial}$ and T_{final} from standard curve

Reaction Time = $T_{final} - T_{initial}$ (minutes)

S_v = sample volume (μL) added to well

CS activity is reported as nmole/min/μL = milliunit/μL.

One unit of citrate synthase is the amount of enzyme that generates 1.0 μmole of CoA per minute at 25 °C and pH 7.2.

Sample Calculation:

Amount of GSH (S_a) = 15.84 nmole (from standard curve)

($T_{initial}$) = 3 minutes

(T_{final}) = 32 minutes

Sample volume (S_v) = 50 μL

CS activity in sample well:

$$\text{nmole/min/μL (milliunits/μL)} = \frac{15.84 \text{ nmole/well}}{(32 \text{ min} - 3 \text{ min}) \times 50 \text{ μL/well}} = 0.0109$$

References

1. Cheng, T.L., et al., Identification and characterization of the mitochondrial targeting sequence and mechanism in human citrate synthase. J. Cell Biochem., **107**, 1002–1015 (2009).
2. Petrohai, A. et al., Detection of citrate synthase-reacting autoantibodies after heart transplantation: an epitope mapping study. Transpl. Int., **17**, 834 – 840 (2005).
3. Crumbley, C. et al., Regulation of expression of citrate synthase by the retinoic acid receptor-related orphan receptor α (RORα). PLoS One., **7**, e33804 (2012).

Troubleshooting Guide

Problem	Possible Cause	Suggested Solution
Assay not working	Cold assay buffer	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 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
	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 needed to use 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 Mixes 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 Mixes
	Pipetting errors in preparation of standards	Avoid pipetting small volumes
	Pipetting errors in the Reaction Mix	Prepare Reaction Mixes 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

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