Fluorescent Sarcosine Detection Kit

Cat #: orb1821125 (manual)

Introduction:

Sarcosine is a natural amino acid that is an important intermediate in the metabolism of choline. Sarcosine is an important component of proteins and plays a significant role in metabolic processes of living cells as a source of serine, creatine, purines and glutathione etc. Sarcosine is present in such food sources like legumes, eggs, ham, turkey etc. It is used in a variety of industrial applications such as manufacturing of toothpaste and biodegradable surfactants.

Sarcosine was recently reported to activate prostate cancer cells and is indicated as a possible marker for prostate cancer progression to metastasis. (2) It is also used in adjunctive treatment for Schizophrenia (3) and depression (4).

Fluoro Sarcosine assay provides a reliable, sensitive fluorimetric assay for the quantification of sarcosine in biological samples.

Applications:

- Detection of sarcosine in cells or tissue extracts.

- Detection sarcosine in serum.

Assay Principle:

The Fluoro Sarcosine detection kit utilizes a non-fluorescent detection reagent, which is reduced via an enzyme-coupled reaction in the presence of sarcosine. A sarcosine standard curve is generated to quantify sarcosine in the samples.

Reaction:

Sarcosine + non-fluorescent detection reagent+ Enzyme Mix $\longrightarrow$ fluorescent analog

Excitation: 530-570nm and Emission at 590-600nm

Storage:

1. **Upon arrival store the following components at -20°C.**

Enzyme Mix.

2. **Upon arrival store the following components between 2 to 8°C.**

Sarcosine detection reagent.

Sarcosine Standard.

Standard curve diluent.

Biorbyt Ltd
7 Signet Court, Swann Road, Cambridge, CB5 8LA. United Kingdom
Email: info@biorbyt.com | Phone: +44 (0)1223 859-353 | Fax: +44 (0)1223 280-240

Biorbyt LLC
68 TW Alexander Drive, Durham, NC 27709. United States
Email: info@biorbyt.com | Phone: +1 (415) 906-5211 | Fax: +1 (415) 651-8558

Warnings and Precautions:

1. **For Research use only. Not for use in diagnostic procedures**.
2. Practice safe laboratory procedures by wearing gloves, protective clothing, and eyewear.
3. The reaction is not stable in the presence of thiols (DTT or 2-mercaptoethanol). Keep these reactants below 10µM.
4. Once the vial of sarcosine detection reagent is reconstituted, it is important that low lighting conditions be used while aliquoting as well as performing the experiment. Direct and prolonged light exposure may increase the background, resulting in compromised linearity.

Kit contents and Storage (for 100 assays):

1. Enzyme Mix: 1 vial. Upon arrival store at -20°C.
2. Standard Curve Diluent 40 mL. Upon arrival store at 2-8°C. Ready to use.
3. Sarcosine detection reagent: 1 vial. Upon arrival store at 2-8°C.
4. Sarcosine standard 1 vial. Storage 2-8°C.

Materials required but not supplied:

1. Black 96-well plates (clear bottom optional for bottom reading instruments).
2. Fluorescence plate reader.
3. Deionized water.
4. 1X PBS
5. NP40 Fluka (optional; see technical note b. section XII).
6. Dry DMSO.
7. Protein Assay kit (optional, if required for normalizing protein concentration.

Tissue Preparation:

See Section XI: Technical notes a and b.

Tissue preparation: Prior to tissue extraction, exsanguinate (optional) the animal to remove red blood cells from tissue. Weigh 40-60 mg of tissue and rinse in ice cold PBS (**Note: if you are going to normalize your samples for protein concentration, e.g., using BCA assay, see technical notes b. Section XI**). Transfer it into a 1.5mL eppendorf tube and add 200µL to 500 µL of standard curve diluent or 1X PBS + 0.1% (see technical note b. section XII) to each tube. Then using standard techniques homogenize the tissue samples on ice. Clarify the homogenate by centrifuging the samples at 8000- 10,000g. Next heat the homogenates at 60^0C for 30 minutes.

If not using the supernatants immediately, freeze at –80^0C.

Note: Each investigator should optimize the mg of tissue used per test. It is better to make a concentrated homogenate and titrate the sample, in standard curve diluent so that the values fall within the standard curve.

Mammalian Cell Preparation:

See Section XI: Technical notes a and b.

1. Adherent cells should be detached first. Then follow step 2 below.
2. Spin down 1 x 10^6 – 10 x 10^6 cells. Decant supernatant (media) and wash cells with 5 mL of PBS twice. After the final wash decant the supernatant and dislodge the cell pellet by gently vortexing.
3. Add 200µL to 500 µL of standard curve diluent (**Note: if you are going to normalize your samples for protein concentration, e.g., using BCA assay, see technical note b. Section XI**) to each tube. Vortex the tubes and allow the cells to lyse for 15 minutes at room temperature. Some cell lines are difficult to lyse. To ensure complete lysis, subject the samples to several freeze and thaw cycles. Clarify the homogenate by centrifuging the samples at 8000- 10,000g. Next heat the lysates at 60^0C for 30 minutes.
4. If not using the supernatants immediately, freeze at –80^0C.

Note: Each investigator should optimize the number of cells used per test. It is better to make a concentrated cell lysate and titrate the sample in standard curve diluent so that the values fall within the standard curve.

Serum Samples:

Serum can be directly diluted in standard curve diluent. Make

≥1:5 dilution of the serum samples. Heat the diluted serum samples at 60^0C for 30 minutes. If not using the samples immediately, freeze at –80^0C.

Note: The samples should be titrated in the Standard curve diluent.

Assay Protocol:

1. **Reconstitution of Reagents.**

A. Reconstitute the enzyme mix with 120µL of sterile Di water. Vortex the vial and allow it to sit at room temperature for 15 minutes. The reconstituted vial should be aliquoted so to prevent freeze and thaw cycles.

B. Sarcosine Standard Curve.

Reconstitute the dried vial of the sarcosine standard with 120µL of anhydrous DMSO. Vortex the vial and allow it to sit at room temperature for 15 minutes. Label suitable tubes 1-8. To tube#1 add 490µL of Standard curve diluent and 10µL of the reconstituted sarcosine standard. This will make a 20µM standard. Next to tubes 2-8 add 250µL of the standard curve diluent. Serially transfer 250µL (1:2) of the 20µM standard (tube #1) to tube #2 Vortex tube #2 and transfer 250µL to tube #3. Continue this process to tube #7. Tube # 8 is the blank control. The table below represents sarcosine concentrations.

Tube #	Sarcosine Concentration in tubes.	Final sarcosine Concentration in wells.
1	20µM	10µM
2	10µM	5µM
3	5µM	2.5µM
4	2.5µM	1.25µM

5	1.25µM	0.625µM
6	0.625µM	0.3125µM
7	0.3125µM	0.1562µM
8	0	0

C. Sarcosine Detection Reagent.

Reconstitute the dried vial with 120µL of anhydrous DMSO. Vortex the vial and allow it to sit at room temperature for 15 minutes. Aliquot the detection reagent into single use vials to prevent freeze thaw cycles and store at -20^{0}C.

D. Preparation of Reaction Cocktail:

To every 0.960mL of Standard curve diluent, add 20µL of enzyme mix and 20µL of reconstituted detection reagent. This is enough for 20 reactions. Make enough reaction cocktail for one day's worth of experimentation.

Note: The reaction cocktail is light sensitive. Avoid direct and prolonged exposure to light, as this will increase background.

ASSAY:

1. Add 50µL of standard or sample in triplicate to individual wells of a black 96 well plate. It is recommended to titrate out the sample, in the standard curve diluent, several fold so its values will fall within the range of the standard curve.
2. Next pipette in 50µL of the reaction cocktail (from step 2 above) to all the wells.
3. Place cover on the plate and incubate at 25^{0}C for 30 minutes.
4. Measure fluorescence with excitation at 530-570 nm and emission at 590-600nm using a fluorescent plate reader.

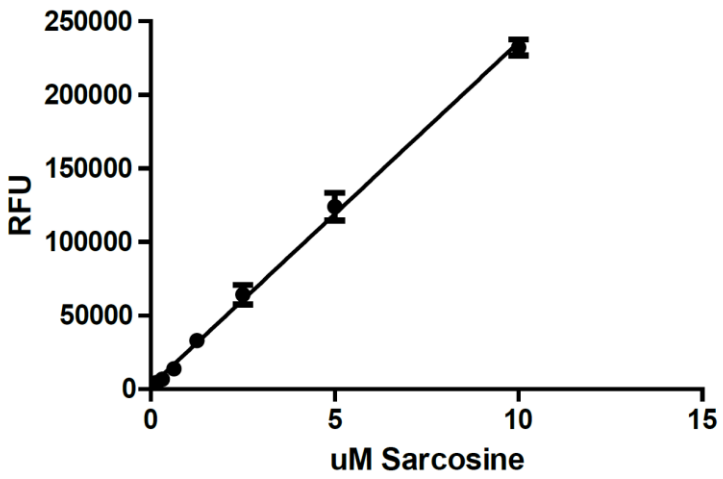

Figure 1. Sarcosine standard curve was generated as described in the protocol. R^2= 0.998

Technical Notes:

a. SH groups like DTT or Reduced Glutathione will interfere with the assay. Keep below 10µM or omit them from the samples.

Note: if you are going to normalize your samples for protein concentration, e.g. using BCA assay. Make a 1X PBS + 0.1% NP40 solution. Use this solution to dilute and lysis your cells or make tissue homogenates.

Spike and recovery results:

Amount Sarcosine spiked in serum	% Recovery
75µM	103
37.5µM	104

Table 1: We conducted spike and recovery experiments to estimate % recovery of sarcosine. Serum was spiked with sarcosine with the concentrations mentioned in the table above. The samples were processed as described in section IX.

Amount Sarcosine spiked in cell lysates	% Recovery
75µM	97.03
37.5µM	98.80.

Table 2: We conducted spike and recovery experiments to estimate % recovery of sarcosine. Cell lysates were spiked with sarcosine with the concentrations mentioned in the table above. The samples were processed as described in section VIII: Mammalian Cell Preparation.

Sample	µM Sarcosine
Serum A	1.529
Serum B	2.092
Serum C	2.486
Jurkats	1.461
Daudi	1.116

Table 3: Serum samples were diluted 1:5 in standard curve diluent. Jurkat and Daudi cells were prepared as described in the protocol. After the final wash cells were adjusted to $1X10^6$ cells/mL in standard curve diluent. Sarcosine was measured as described in the protocol.

Biorbyt Ltd
7 Signet Court, Swann Road, Cambridge, CB5 8LA. United Kingdom
Email: info@biorbyt.com | Phone: +44 (0)1223 859-353 | Fax: +44 (0)1223 280-240

Biorbyt LLC
68 TW Alexander Drive, Durham, NC 27709. United States
Email: info@biorbyt.com | Phone: +1 (415) 906-5211 | Fax: +1 (415) 651-8558