ab204718

Succinate Assay Kit (Colorimetric)

Instructions for Use

For rapid, sensitive and accurate measuring of Succinate.

This product is for research use only and is not intended for diagnostic use.

PLEASE NOTE: With the acquisition of BioVision by Abcam, we have made some changes to component names and packaging to better align with our global standards as we work towards environmental-friendly and efficient growth. You are receiving the same high-quality products as always, with no changes to specifications or protocols.

Version 5a, Last Updated 2 June 2023

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1. BACKGROUND

Succinate Assay Kit (Colorimetric) (ab204718) is a sensitive, fast and easy-to-use kit. In this assay, succinate is utilized by Succinyl-CoA Synthetase to form an intermediate, which undergoes a series of reactions and reduces a colorless probe to a colored product with strong absorbance at 450 nm. This assay kit can detect less than 40 µM of succinate or succinic acid in a variety of sample types.

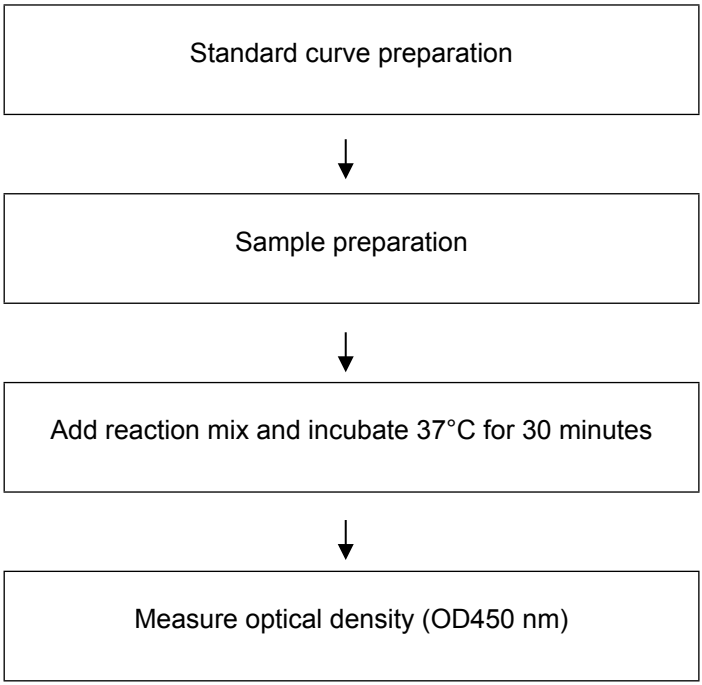
Succinate + ATP + CoA converted by succiny-CoA synthase to succiny-CoA + ADP + Pi with Color Detection (OD 450 nm)

Succinate ($C_4H_4O_4$) is an intermediate in the Krebs / citric acid cycle and is capable of donating electrons to the electron transport chain by its conversion to fumarate in a reaction catalyzed by Succinate dehydrogenase (or Complex II).

Succinate is the salt or ester of succinic acid. Succinic acid ($C_4H_6O_4$) is distributed in all plants and animal tissues and was first obtained from amber. Due to its low toxicity, it is widely used in agriculture, food and pharmaceutical industry. Succinic acid concentrations are monitored in the manufacture of numerous foodstuffs and beverages such as wine, soy sauce, fruit juice and dairy products.

Measurement of succinate or succinic acid level is important a key to analysis of the citric acid cycle.

2. ASSAY SUMMARY



3. PRECAUTIONS

Please read these instructions carefully prior to beginning the assay.

All kit components have been formulated and quality control tested to function successfully as a kit. Modifications to the kit components or procedures may result in loss of performance.

4. STORAGE AND STABILITY

Store kit at -20ºC in the dark immediately upon receipt. Kit has a storage time of 1 year from receipt, providing components have not been reconstituted.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in Materials Supplied section.

Aliquot components in working volumes before storing at the recommended temperature. **Reconstituted components are stable for 2 months.**

5. LIMITATIONS

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

6. MATERIALS SUPPLIED

Item	Amount	Storage Condition (Before Preparation)	Storage Condition (After Preparation)
Assay Buffer VII/Succinate Assay Buffer	25 mL	-20°C	-20°C
Succinate Converter/Succinate Converter (Lyophilized)	1 vial	-20°C	-20°C
Enzyme Mix XI/Succinate Enzyme Mix (Lyophilized)	1 vial	-20°C	-20°C
Succinate Substrate Mix/Succinate Substrate Mix (Lyophilized)	1 vial	-20°C	-20°C
Developer Solution III/Succinate Developer (Lyophilized)	1 vial	-20°C	-20°C
Succinate Standard/Succinate Standard (Lyophilized)	1 vial	-20°C	-20°C

7. MATERIALS REQUIRED, NOT SUPPLIED

These materials are not included in the kit, but will be required to successfully perform this assay:

- MilliQ water or other type of double distilled water (ddH_2O)
- PBS
- Microcentrifuge
- Pipettes and pipette tips
- Colorimetric microplate reader – equipped with filter for OD 450 nm
- 96 well plate: clear plate with flat bottom
- Heat block or water bath
- Dounce homogenizer or pestle (if using tissue)

For liquid samples:

- Polyvinylpyrrolidone (PVPP) - for liquid samples with strong color
- 10 kD Spin Columns (ab93349)
- (Optional) 0.5M Tris HCl, pH 8.0 – to neutralize acidic samples

8. TECHNICAL HINTS

- **This kit is sold based on number of tests. A 'test' simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.**
- Selected components in this kit are supplied in surplus amount to account for additional dilutions, evaporation, or instrumentation settings where higher volumes are required. They should be disposed of in accordance with established safety procedures.
- Keep enzymes, heat labile components and samples on ice during the assay.
- Make sure all buffers and solutions are at room temperature before starting the experiment.
- Samples generating values higher than the highest standard should be further diluted in the appropriate sample dilution buffers.
- Avoid foaming or bubbles when mixing or reconstituting components.
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions.
- Ensure plates are properly sealed or covered during incubation steps.
- Make sure you have the right type of plate for your detection method of choice.
- Make sure the heat block/water bath and microplate reader are switched on.
- This kit has so far only been tested with human and rat samples. Since Succinate is distributed in all plants and animal tissues this kit should detect Succinate independent of the species. Depending on the sample type, the sample preparation might need to be optimized.

9. REAGENT PREPARATION

- Briefly centrifuge small vials at low speed prior to opening.

9.1 **Assay Buffer VII/Succinate Assay Buffer:**

Ready to use as supplied. Equilibrate to room temperature before use. Store at -20°C.

9.2 **Succinate Standard:**

Reconstitute the Succinate Standard in 100 µL dH_2O to generate a 100 mM (100 nmol/µL) Succinate Standard stock solution. Aliquot standard so that you have enough volume to perform the desired number of assays. Store at -20°C. Keep on ice while in use.

9.3 **Succinate Converter:**

Reconstitute in 220 µL Assay Buffer. Aliquot converter so that you have enough volume to perform the desired number of assays. Store at -20°C. Keep on ice while in use. Use within two months.

9.4 **Enzyme Mix XI/Succinate Enzyme Mix:**

Reconstitute in 220 µL Assay Buffer. Aliquot Enzyme Mix so that you have enough volume to perform the desired number of assays. Store at -20°C. Keep on ice while in use. Use within two months.

9.5 **Succinate Substrate Mix:**

Reconstitute in 220 µL Assay Buffer. Aliquot Substrate Mix so that you have enough volume to perform the desired number of assays. Store at -20°C. Keep on ice while in use. Use within two months.

9.6 **Developer Solution III/Succinate Developer:**

Reconstitute in 220 µL dH_2O. Aliquot developer so that you have enough volume to perform the desired number of assays. Store at -20°C. Use within two months.

10. STANDARD PREPARATION

- Always prepare a fresh set of standards for every use.
- Diluted standard solution is unstable and cannot be stored for future use.

10.1 Prepare a 1 mM (1 nmol/µL) Succinate standard by diluting 10 µL of the reconstituted 100 mM Succinate standard with 990 µL of ddH_2O.

10.2 Using 1 mM Succinate standard, prepare standard curve dilution as described in the table in a microplate:

Standard #	Volume of Standard (µL)	Assay Buffer (µL)	Final volume standard in well (µL)	End Amount Succinate in well (nmol/well)
1	0	150	50	0
2	6	144	50	2
3	12	138	50	4
4	18	132	50	6
5	24	126	50	8
6	30	120	50	10

Each dilution has enough amount of standard to set up duplicate readings (2 x 50 µL).

11. SAMPLE PREPARATION

General Sample information:

- We recommend performing several dilutions of your sample to ensure the readings are within the standard value range.
- We recommend that you use fresh samples. If you cannot perform the assay at the same time, we suggest that you complete the Sample Preparation step as well as the deproteinization step before storing the samples. Alternatively, if that is not possible, we suggest that you snap freeze cells or tissue in liquid nitrogen upon extraction and store the samples immediately at -80°C. When you are ready to test your samples, thaw them on ice. Be aware however that this might affect the stability of your samples and the readings can be lower than expected.

11.1 Cell (adherent or suspension) samples:

11.1.1 Harvest the amount of cells necessary for each assay (initial recommendation = 1 x 10^6 cells).

11.1.2 Wash cells with cold PBS.

11.1.3 Resuspend cells in 100 µL of ice cold Assay Buffer VII/Succinate Assay Buffer.

11.1.4 Rapidly homogenize cells quickly by pipetting up and down a few times on ice.

11.1.5 Centrifuge sample for 5 minutes at 4°C at top speed using a cold microcentrifuge to remove any insoluble material.

11.1.6 Collect supernatant and spin filter by using 10 kDa spin column (ab93349).

11.1.7 Keep on ice.

11.2 **Tissue samples:**

11.2.1 Harvest the amount of tissue necessary for each assay (initial recommendation = 10 mg).

11.2.2 Wash tissue in cold PBS.

11.2.3 Resuspend tissue in 100 µL of ice cold Assay Buffer VII/Succinate Assay Buffer.

11.2.4 Rapidly homogenize tissue with a Dounce homogenizer sitting on ice, with 10 – 15 passes.

11.2.5 Centrifuge samples for 5 minutes at 4°C at top speed using a cold microcentrifuge to remove any insoluble material.

11.2.6 Collect supernatant and spin filter by using 10 kDa spin column (ab93349).

11.2.7 Keep on ice.

11.3 **Liquid Samples:**

For liquid samples having strong color, we recommend to use polyvinylpyrrolidone (PVPP) to remove the color.

For acidic samples (e.g. white wine), neutralize the sample (1:1 dilution) with 0.5 M Tris HCl, pH 8.0.

11.3.1 Mix sample with 1% PVPP (w/v) for 5 minutes at room temperature.

11.3.2 Spin filter the samples by using 10 kDa spin column (ab93349).

NOTE: *We suggest using different volumes of sample to ensure readings are within the Standard Curve range.*

12. ASSAY PROCEDURE and DETECTION

- **Equilibrate all materials and prepared reagents to room temperature prior to use.**
- **It is recommended to assay all standards, controls and samples in duplicate.**

12.1 **Set up Reaction wells:**

- Standard wells = 50 µL standard dilutions.
- Sample wells = 1 – 50 µL samples (adjust volume to 50 µL/well with Assay Buffer).
- Background control sample wells= 1 – 50 µL samples (adjust volume to 50 µL/well with Assay Buffer). ***NOTE:*** *NADH in samples will generate background. For samples having high NADH levels, prepare parallel sample well(s) as background control.*

12.2 **Reaction Mix:**

Prepare 50 µL of Reaction Mix for each reaction

Component	Reaction Mix (µL)	Background Control Mix (µL)
Assay Buffer VII/Succinate Assay Buffer	**42**	**44**
Succinate Converter	**2**	**0**
Enzyme Mix XI/Succinate Enzyme Mix	**2**	**2**
Succinate Substrate Mix	**2**	**2**
Developer Solution III/Succinate Developer	**2**	**2**

Mix enough reagents for the number of assays (samples and controls) to be performed. Prepare a master mix of the Reaction Mix to ensure consistency. We recommend the following calculation: X µL component x (Number reactions +1).

12.3 Add 50 µL of appropriate Reaction Mix into each standard and sample wells.

12.4 Add 50 µL of Background Reaction Mix to Background control sample wells.

12.5 Mix and incubate at 37°C for 30 minutes protected from light.

12.6 Measure output at OD = 450 nm on a microplate reader.

13. CALCULATIONS

- Samples producing signals greater than that of the highest standard should be further diluted in appropriate buffer and reanalyzed, then multiplying the concentration found by the appropriate dilution factor.
- For statistical reasons, we recommend each sample should be assayed with a minimum of two replicates (duplicates).

13.1 Average the duplicate reading for each standard and sample.

13.2 If the sample background control is significant, then subtract the sample background control from sample reading.

13.3 Subtract the mean absorbance value of the blank (Standard #1) from all standard and sample readings. This is the corrected absorbance.

13.4 Plot the corrected absorbance values for each standard as a function of the final concentration of Succinate.

13.5 Draw the best smooth curve through these points to construct the standard curve. Most plate reader software or Excel can plot these values and curve fit. Calculate the trendline equation based on your standard curve data (use the equation that provides the most accurate fit).

13.6 Concentration of Succinate (nmol/µL or µmol/ml or mM) in the test samples is calculated as:

$$Succinate = \left(\frac{A}{B}\right) * D$$

Where:

A = Amount of Succinate (Succinic Acid) from the Standard Curve (nmol).

B = Sample volume added into the reaction well (µL).

D = Sample dilution factor.

Succinic Acid molecular weight: 118.09 g/mol

Sample Succinate or Succinic Acid concentration can also be expressed in nmol/mg sample.

14. TYPICAL DATA

TYPICAL STANDARD CURVE – Data provided for **demonstration purposes only**. A new standard curve must be generated for each assay performed.

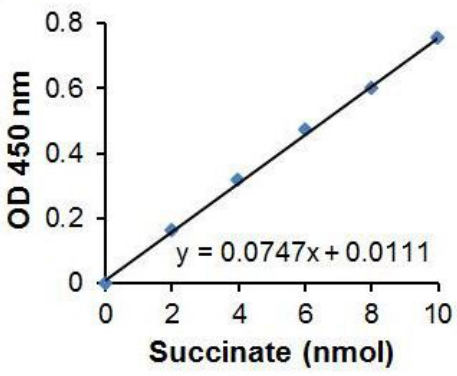


Figure 1. Typical Succinate Standard calibration curve.

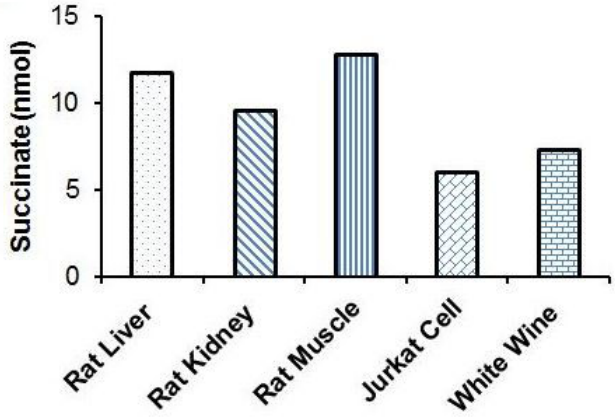


Figure 2. Measurement of Succinate levels in rat liver (40 µg), kidney (40 µg) and muscle (40 µg), Jurkat cell lysate (200 µg) and white wine (4 µL, wine sample was diluted 1:1 with 0.5 M Tris HCl, pH 8.0).

15. QUICK ASSAY PROCEDURE

***NOTE**: This procedure is provided as a quick reference for experienced users. Follow the detailed procedure when performing the assay for the first time.*

- Prepare standard, Succinate convertor, Developer Solution III/Succinate developer and prepare enzyme mix (aliquot if necessary); get equipment ready.
- Prepare standard curve.
- Prepare samples in duplicate (find optimal dilutions to fit standard curve readings).
- Set up plate for standard (50 µL), samples (50 µL) and background wells (50 µL).
- Prepare Succinate Reaction Mix (Number samples + standards + 1).

Component	Reaction Mix (µL)	Background Control Mix (µL)
Assay Buffer VII/Succinate Assay Buffer	**42**	**44**
Succinate Converter	**2**	**-**
Enzyme Mix XI/Succinate Enzyme Mix	**2**	**2**
Succinate Substrate Mix	**2**	**2**
Developer Solution III/Succinate Developer	**2**	**2**

- Add 50 µL of Succinate Reaction Mix to the standard and sample wells.
- Add 50 µL of Background Control Mix to the background control well.
- Incubate plate at 37°C 30 minutes protected from light.
- Measure plate at OD 450 nm.

16. TROUBLESHOOTING

Problem	Cause	Solution
Assay not working	Use of ice-cold buffer	Buffers must be at room temperature
	Plate read at incorrect wavelength	Check the wavelength and filter settings of instrument
	Use of a different 96-well plate	Colorimetric: Clear plates Fluorometric: black wells/clear bottom plate
Sample with erratic readings	Samples not deproteinized (if indicated on protocol)	Use PCA precipitation protocol for deproteinization
	Cells/tissue samples not homogenized completely	Use Dounce homogenizer, increase number of strokes
	Samples used after multiple free/ thaw cycles	Aliquot and freeze samples if needed to use multiple times
	Use of old or inappropriately stored samples	Use fresh samples or store at -80°C (after snap freeze in liquid nitrogen) till use
	Presence of interfering substance in the sample	Check protocol for interfering substances; deproteinize samples
Lower/ Higher readings in samples and Standards	Improperly thawed components	Thaw all components completely and mix gently before use
	Allowing reagents to sit for extended times on ice	Always thaw and prepare fresh reaction mix before use
	Incorrect incubation times or temperatures	Verify correct incubation times and temperatures in protocol

Problem	Cause	Solution
Standard readings do not follow a linear pattern	Pipetting errors in standard or reaction mix	Avoid pipetting small volumes (< 5 µL) and prepare a master mix whenever possible
	Air bubbles formed in well	Pipette gently against the wall of the tubes
	Standard stock is at incorrect concentration	Always refer to dilutions on protocol
Unanticipated results	Measured at incorrect wavelength	Check equipment and filter setting
	Samples contain interfering substances	Troubleshoot if it interferes with the kit
	Sample readings above/ below the linear range	Concentrate/ Dilute sample so it is within the linear range

17. FAQ

Q: What is the standard in this kit made of? What is its source?

A: The standard is a lipid and does not have an animal source. The exact details are proprietary and cannot be disclosed.

Q: Can a protein assay be used to normalize the loading amount into each well?

A: Yes, a protein assay can be run with the homogenate/lysate to normalize the loading amount in each well. We suggest using a BCA assay.

18. INTERFERENCES

19. NOTES

Technical Support

For all technical or commercial enquiries please go to:

www.abcam.com/contactus

www.abcam.cn/contactus (China)

www.abcam.co.jp/contactus (Japan)