



Succinic Acid (K-SUCC) Procedure for ChemWell®-T Auto-Analyser

Requirements:

- Succinic Acid Assay Kit (**K-SUCC**) (provides ~ 270 assays).
- K-SUCC ChemWell®-T assay file.
- Use in association with the Succinic Acid Assay Kit (K-SUCC) product data booklet.

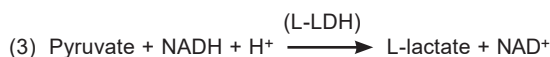
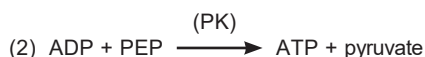
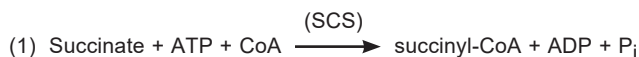
Use:

For the specific measurement of succinic acid especially in wines, beverages, dairy and other food products.

For specific sample preparation methods refer to the Succinic Acid Assay Kit (K-SUCC) data booklet.

Assay Principle:

Conversion of succinate via the following reactions is directly proportional to the coupled consumption of NADH:



Procedure:

Prepare the assay reagents and calibrators and use with the K-SUCC ChemWell®-T assay file.

Succinic Acid Assay Kit Components:

Bottle 1: Buffer (8 mL, pH 8.4) plus sodium azide (0.02% w/v) as a preservative.
Stable for > 2 years at 4°C.

Bottle 2: (x2) NADH plus stabiliser.
Stable for > 2 years below -10°C.

Bottle 3: (x2) ATP plus PEP and CoA.
Stable for > 2 years below -10°C.

Bottle 4: Pyruvate kinase plus L-lactate dehydrogenase suspension (0.55 mL).
Stable for > 2 years at 4°C.

Bottle 5: Succinyl-CoA synthetase suspension (0.55 mL).
Stable for > 2 years at 4°C.

Preparation of Kit Components:

1. Use the contents of bottle 1 as supplied.
Stable for > 2 years at 4°C.
2. Dissolve the contents of one bottle 2 in 2.4 mL of distilled water. **Stable for > 1 year at 4°C** or > 2 years below -10°C (to avoid repetitive freeze / thaw cycles, divide into appropriately sized aliquots and store in polypropylene tubes).
Do not dissolve the contents of the other bottle until required.
3. Dissolve the contents of one bottle 3 in 2.4 mL of distilled water. Divide into appropriately sized aliquots and store in polypropylene tubes below -10°C.
Do not dissolve the contents of the other bottle until

required. Once dissolved the reagent is stable for > 4 weeks below -10°C.

- 4&5.** Use contents of bottles 4 and 5 as supplied. Before opening for the first time, shake the bottles to remove any enzyme that may have settled on the rubber stopper. Subsequently, store the bottles in an upright position. **Swirl the bottle to mix contents before use.**
Stable for > 2 years at 4°C

Preparation of Assay Reagents: (per ~ 140 assays)

Reagent 1:

Component	Volume
distilled water	20.55 mL
bottle 1 (buffer)	2.40 mL
*bottle 2 (NADH)	2.40 mL
*bottle 3 (ATP/PEP/CoA)	2.40 mL
bottle 4 (PK/L-LDH)	0.24 mL
Total volume	27.99 mL

*after adding 2.4 mL of distilled water

Reagent 1 stability: > 2 days at 4°C

Reagent 2:

Component	Volume
distilled water	3.55 mL
bottle 5 (SCS)	0.26 mL
Total volume	3.81 mL

Reagent 2 stability: > 2 days at 4°C

Calibrators:

K-SUCC 1: 0 g/L (use distilled water)
K-SUCC 2: 0.1 g/L succinic acid
K-SUCC 3: 0.2 g/L succinic acid
K-SUCC 4: 0.4 g/L succinic acid

Assay Parameters:

Assay volumes: Reagent 1: 0.200 mL
Sample: 0.01 mL
Reagent 2: 0.025 mL

Calibrators: 0, 0.1, 0.2, 0.4 g/L succinic acid
Reaction time: 6 min at 37°C
Wavelength: 340 nm
Assay type: endpoint
Reaction direction: decrease
Linearity: up to 0.4 g/L of succinic acid.

