



# L-Ascorbic Acid (K-ASCO) Procedure for ChemWell®-T Auto-Analyser

## Requirements:

- Ascorbic Acid (L-Ascorbate) Assay Kit (K-ASCO) (provides ~ 400 assays).
- K-ASCO (BLANK) and K-ASCO (SAMPLE) ChemWell®-T assay files and the K-ASCO (ASCORBIC) ChemWell®-T indices file.
- Use in association with the Ascorbic Acid (L-Ascorbate) Assay Kit (K-ASCO) product data booklet.

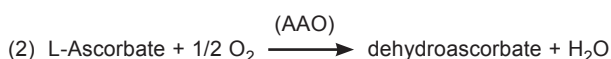
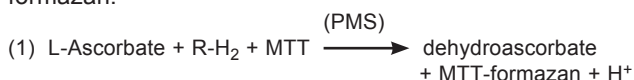
## Use:

For the specific measurement of L-ascorbic acid especially in beverage, fruit, vegetable, dairy, food and feed products.

For specific sample preparation methods refer to the Ascorbic Acid (L-Ascorbate) Assay Kit (K-ASCO) data booklet.

## Assay Principle:

Conversion of L-ascorbic acid (L-ascorbate) via the following reactions is measured using the coupled formation of MTT-formazan:



## Procedure:

Prepare the assay reagents and calibrators and use with the K-ASCO (BLANK) and K-ASCO (SAMPLE) ChemWell®-T assay files and the K-ASCO (ASCORBIC) ChemWell®-T indices file.

## Ascorbic Acid (L-Ascorbate) Assay Kit Components:

- Bottle 1:** Buffer (44 mL, pH 5.6).  
Stable for > 2 years at 4°C.
- Bottle 2:** MTT (18 mL, pH 3.5).  
Stable for > 2 years in the dark at room temperature.
- Bottle 3: (x2)** PMS.  
Stable for > 2 years in the dark at 4°C.
- Bottle 4:** Ascorbic acid oxidase suspension (0.85 mL).  
Stable for > 2 years at 4°C.
- Bottle 5:** L-Ascorbic acid (vitamin C) (~ 2 g).  
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. Use the contents of bottle 2 as supplied.  
Stable for > 2 years in the dark at room temperature.
3. Dissolve the contents of one of bottle 3 in 10.8 mL of distilled water.  
Stable for > 6 months at 4°C
4. Use the contents of bottle 4 as supplied.  
Swirl the bottle to mix contents before use.  
Stable for > 2 years at 4°C.
5. To prepare the K-ASCO 4 calibrator 0.28 g/L accurately weigh 280 mg of L-ascorbic acid into a 1 L volumetric flask. Fill to the mark with 3% (w/v) metaphosphoric acid / 10 mM EDTA buffer and mix thoroughly.  
Stable for > 2 weeks at 4°C.  
Use serial dilutions of K-ASCO 4 in 3% (w/v) metaphosphoric acid / 10 mM EDTA buffer to prepare K-ASCO 2 and K-ASCO 3.

## Preparation of Assay Reagents: (per ~ 200 assays)

### K-ASCO R1-BLANK:

Component	Volume
distilled water	28.5 mL
solution 1 (buffer)	10.0 mL
solution 2 (MTT buffer)	4.0 mL
suspension 4 (AAO)	0.4 mL
Total volume	42.9 mL

K-ASCO R1-BLANK stability: > 2 days at 4°C

### K-ASCO R1-SAMPLE:

Component	Volume
distilled water	28.9 mL
bottle 1 (buffer)	10.0 mL
bottle 2 (MTT buffer)	4.0 mL
Total volume	42.9 mL

K-ASCO R1-SAMPLE stability: > 2 days at 4°C

### K-ASCO R2:

Component	Volume
bottle 3 (PMS)	10.8 mL
Total volume	10.8 mL

\*after adding 10.8 mL of distilled water

K-ASCO R2 stability: > 2 days at 4°C

## Calibrators:

- K-ASCO 1: 0 g/L (use distilled water)  
K-ASCO 2: 0.07 g/L ascorbic acid  
K-ASCO 3: 0.14 g/L ascorbic acid  
K-ASCO 4: 0.28 g/L ascorbic acid

## Assay Parameters:

### Assay volumes: K-ASCO (BLANK)

K-ASCO R1-BLANK:	0.200 mL
Sample:	0.010 mL
K-ASCO R2:	0.025 mL

### Assay volumes: K-ASCO (SAMPLE)

K-ASCO R1-SAMPLE:	0.020 mL
Sample:	0.010 mL
K-ASCO R2:	0.025 mL

- Calibrators: 0, 0.07, 0.14, 0.28 g/L ascorbic acid  
Reaction time: 8 min at 37°C  
Wavelength: 580 nm  
Assay type: endpoint  
Reaction direction: increase  
Linearity: up to 0.28 g/L ascorbic acid

## ChemWell®-T Files:

- K-ASCO (BLANK) measures reducing substances after selectively removing L-ascorbic acid (g/L).
- K-ASCO (SAMPLE) measures total reducing substances (g/L).
- K-ASCO (ASCORBIC) indices file calculates L-ascorbic acid (g/L) from K-ASCO (BLANK) and K-ASCO (SAMPLE).

