

K-ASCO FAQs (10/05)

Q1. What absorbance value should be attained using the ascorbic acid standard supplied with K-ASCO?

A. When the L-ascorbic acid standard is made up as described in the K-ASCO data booklet (0.15 g/L and 0.1 mL used in the assay) the expected absorbance value should be approximately 0.57 above the blank value when tested using the standard K-ASCO procedure.

Q2. Is it possible to adapt the kit for use on various auto-analysers?

A. Yes. If the assay format is not available for your auto-analyser you can supply the following parameters of your relevant auto-analyser to Megazyme and an appropriate assay format may be available:

Parameters:

Instrument make and model

Light-path length

Reagent addition volumes (e.g. R1 volume, R2 volume, R3 if applicable)

Suggested auto-analyser format for K-ASCO:

Prepare the reagents as described in the K-ASCO databooklet then prepare R1 and R2 as follows:

Preparation of R1: (90 assays)

Component	Volume
Bottle 1	5.6 mL
Bottle 4	0.225 mL
Distilled water	17.5 mL
Total volume	23.325 mL

Preparation of R2: (90 assays)

Component	Volume
Bottle 2	2.25 mL
Bottle 3	2.25 mL
Total volume	4.5 mL

EXAMPLE METHOD:

R1: 0.260 mL

Sample: ~ 0.005 mL

R2: 0.05 mL

Note: For accurate measurement of ascorbic acid each sample requires two measurements, one with AAO (bottle 4) present and one with AAO (bottle 4) absent.

In the auto-analyser format described above there is no option to read A1 as in the cuvette assay. In this instance use an ascorbic acid calibration curve to calculate results. Alternatively add 0.025 mL of bottle 2 and 3 separately to mimic the cuvette assay and allow the reading A1 (this will require 3 reagent additions) OR it may be possible to add the MTT (bottle 2) with R1 as follows:

Preparation of R1: (90 assays)

Component	Volume
Bottle 1	5.6 mL

Bottle 2	2.25 mL
Bottle 4	0.225 mL
Distilled water	17.5 mL
Total volume	25.575 mL

Preparation of R2: (90 assays)

Component	Volume
Bottle 3	2.25 mL
Total volume	2.25 mL

EXAMPLE METHOD:

R1: 0.285 mL

Sample: ~ 0.005 mL

R2: 0.025 mL

Q3. Is it possible to use K-ASCO to measure ascorbic acid in fruits and what extraction procedure is recommended?

A. Yes. For solid fruits the following procedure is recommended.

Accurately weigh approximately 4 g of sample and homogenise in 10 mL of 1M potassium phosphate buffer (pH 3.5) with an Ultraturrax® or Polytron® homogeniser (or equivalent) to effect complete disintegration. Adjust the pH to 3.5-4.0, if necessary, with 2M KOH and quantitatively transfer the slurry to a 100 mL volumetric flask. Rinse the homogeniser shaft with water and add these washings to the flask. Fill to the mark with 100 mM potassium phosphate buffer (pH 3.5) then filter through Whatman No.1 filter paper or centrifuge at 13000 g. Use the filtrate/supernatant in the assay with an appropriate dilution in distilled water if required.

If coloured solutions require analysis undiluted, they may need decolorising as follows: stir 10 mL of liquid sample for 5 min with 0.2 g of PVPP and then filter through Whatman No.1 filter paper or centrifuge at 13000 g. Use the clear/slightly coloured filtrate/supernatant directly in the assay.

Ascorbic acid extracts may be stabilised by the addition of *tert*-Butylhydroquinone (TBHQ, tertiary butylhydroquinone) at a final concentration of 1mM.