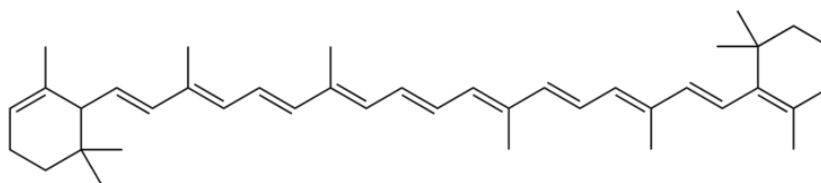


BETA-CAROTENE IN PLASMA BY UV/VIS - FAST – Code Z64010

BIOCHEMISTRY

Carotene is a provitamin terpene contained in many fruits and vegetables to give them a color ranging from yellow to red. It is present particularly in rich fruits such as apricots, persimmons, melons, peaches, oranges and vegetables like carrots, tomatoes, yellow squash, red peppers, but also green leafy vegetables such as spinach, broccoli, the turnips and chicory. It consists of 8 isoprene units cyclization to extremes and exists in two forms called alpha and beta (see illustration). Beta-carotene is the most common form in nature. Arriving in the gut, it is converted in part into vitamin A, a substance essential for healthy skin and eyes, and the rest is absorbed and stored.



EUREKA S.R.L. LAB DIVISION
Via M. D'Antona 28
60033 Chiaravalle (AN) - Italy
Tel. +39 071 7450790
eureka-support@sentinel.it
www.eurekakit.com



This product fulfills all the requirements of Directive 98/79/EC on in vitro diagnostic medical devices (IVD). The declaration of conformity is available upon request.

N° 007	Beta-Carotene in plasma by UV/VIS - FAST	11/2025
--------	--	---------

TECHNICAL FEATURES

Principle of the Method :

This method allows to determine plasma concentrations of **beta-carotene**. A rate of two reagents are added plasma precipitation and, after centrifugation, the supernatant is transferred into the test tube and injected into HPLC.

Recovery :

> 95%

Minimum concentration analyzed:

30 µg/L

Dynamic Range:

30 – 10.000 µg/L

Normal Range :

beta-Carotene : 100 – 600 µg/L

Components of the kit :

All the Reagents are stable 3 years at 2 –8 °C, except for Reagent A and F.
Reagent F is stable 2 months at **-20 °C**

Reagent A – Deproteinization Solution with Internal Standard, **1 x 60 ml** **Store at -20 °C**

Reagent B – Precipitant Solution, **1 x 100 ml**

Reagent C – Stabilizing Solution, **1 x 10 ml**

Reagent F – Test Solution/Chemical Standard, **1 x 10 ml** **See Warnings - Store at -20 °C**

Reagent M – Mobile Phase, **2 x 500 ml**

Minimum Instrumental equipment required:

Isocratic HPLC System with loop of 20 µl
Spectrophotometric Detector UV/VIS $\lambda = 453 \text{ nm}$
Chromatograms Recorder

Optional Equipment:

Autosampler
Operational Computer

Blood Collection Procedure :

Take 3 ml of blood in a tube with **citrate** as anticoagulant.

Pre-analytical Phase :

If you are not analyzed immediately, freeze the sample at -20 °C. Stable for 3 months.
Stable 1 week at 2-8 °C.

PREANALYTICAL PROCEDURE

Inject 20 µl of **Reagent F – Test Solution 1000 µg/L** and verify that the beta-Carotene has retention time similar between 6 and 8 minutes. If the Test is all right you can start with the analytical procedure; if not, check the functionality of the analytical system.

ANALYTICAL PROCEDURE

STEP 1 : Samples Preparation

Dispense in ***strictly glass tubes***:

	Calibrator	Sample
Reagent F – Chemical Standard	400 µl	
Sample		400 µl
Reagent A – Deproteinization Sol.	600 µl	600 µl

Warning: deproteinize directly on Vortex for at least 30 sec.

STEP 2 : Add 1 ml of **Reagent B – Precipitant Solution**

Vortex for 20 sec.

STEP 3 : Centrifuge at 4.000 rpm for 10 minutes.

STEP 4 : Place the supernatant in a clean ***strictly glass tubes***.

STEP 5 : To dryness under a gentle flow of helium or nitrogen and resume with 100 µl of **Reagent C – Stabilizing Solution**

N.B.: at this step the sample is stable 2 days at 2-8 °C

Vortex for 5 sec.

INJECTION :

- Inject 20 µl of the solution into the chromatographic system.

BETA-CAROTENE IN PLASMA BY UV/VIS - FAST – Warnings

REAGENT F : TEST SOLUTION / CHEMICAL STANDARD

Beta-Carotene	1000 µg/L
Stable 2 months at -20 °C	

SPECTROPHOTOMETRIC DETECTOR PARAMETERS

λ	453 nm
GAIN	0,001 AUFS
INTEGRATION TIME	10 sec

HPLC COLUMN PROTECTION

To save the analytical column Reverse Phase POROSHELL EC C18 (50 x 4,6 mm – 2,7 µm), the use of Javelin Prefilters (1 x 10 pcs), code S90199511 is obligatory.
--

HPLC COLUMN CONDITIONING

Install a <u>new</u> analytical column Reverse Phase POROSHELL EC C18 (50 x 4,6 mm – 2,7 µm). Disconnect the detector and flush 30 ml of a solution of H₂O : Methanol (20 : 80 v/v) set flow at 1,0 ml / min. Don't recycle the washing solutions. Flush 30 ml of a solution of H₂O : Methanol (80 : 20 v/v) set flow at 1,0 ml / min. Condition the column with the mobile phase at a flow of 1ml / min. discharge first 20 ml. Condition further on the column for 30 min. also at recycling phase. Finally inject the derivatized Chemical Standard and verify the quality of the HPLC run. It is possible to make analysis at recycling phase. If room temperature is > 20 °C store the Mobile Phase at 2-8 °C between an analytical session and another.

COLUMN CLEANING

Disconnect the detector. Flush 15 ml of H ₂ O: methanol or Acetonitrile (80: 20 v/v) and set flow at 1,0 ml / min. Flush a solution of H ₂ O: methanol or Acetonitrile (70: 30 v/v) for 30 minutes for download. When the column will be used again, pass 15 ml of H ₂ O: methanol (20: 80 v/v) before made conditional with the Mobile phase. It is recommended to wash the analytical column at the end of each session. The column is stored in a solution of H₂O : Acetonitrile or Methanol (30 : 70 v / v)
--

INJECTION NEEDLE WASH

Wash in a solution of H ₂ O / Ethanol or Methanol or Acetonitrile (80 : 20 v/v).

HPLC PARAMETERS

LOOP	20 µl
Recommended Flow rate	1,5 ml/min
Pressure	About 80 bar

ACCESSORIES AND CONSUMABLES

CODE	DESCRIPTION	PACKAGING
Z64020	Chemical Standard for beta-Carotene	2 x 5 ml
Z699975902	Poroshell 120, EC-C18 (50 x 4,6 mm - 2,7 µm) Analytical Column	1 Pc
S90199511	Javelin Colfilters	1 x 10 Pcs
S51843550	Clear glass vials with reduced volume from 1,5 ml to 15 µl	1 x 100 Pcs
S51820717	Caps for Clear glass vials with reduced volume from 1,5 ml to 15 µl	1 x 100 Pcs

Beta-CAROTENE IN PLASMA

(Reference Chromatograms)

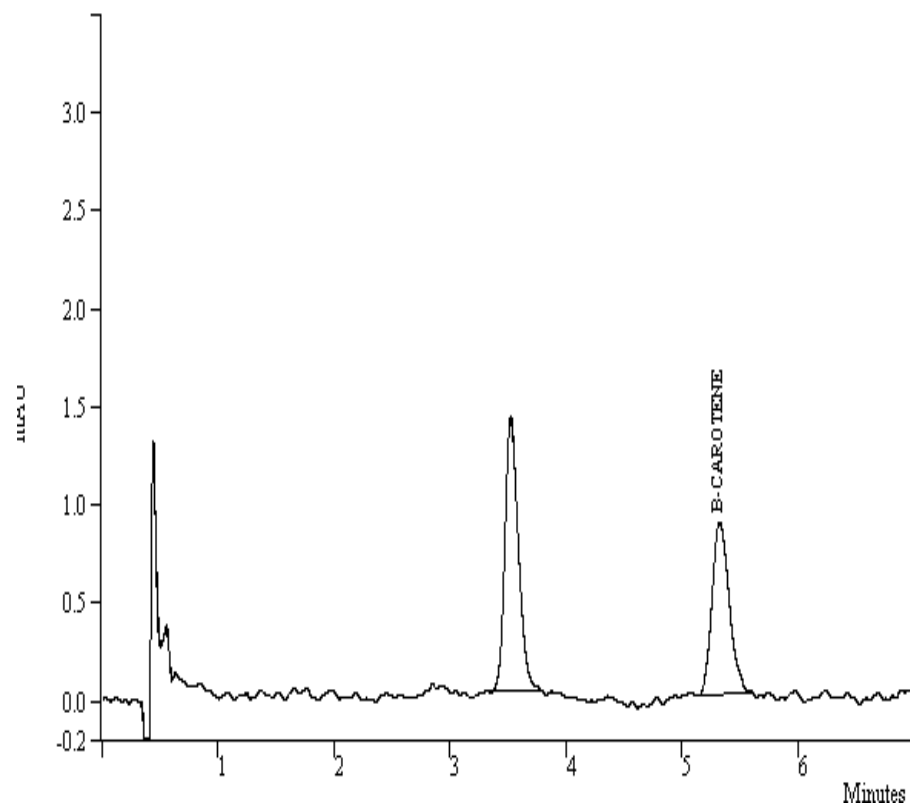


Fig. 1 :	Test Solution
	R.T. 5.3 beta-Carotene

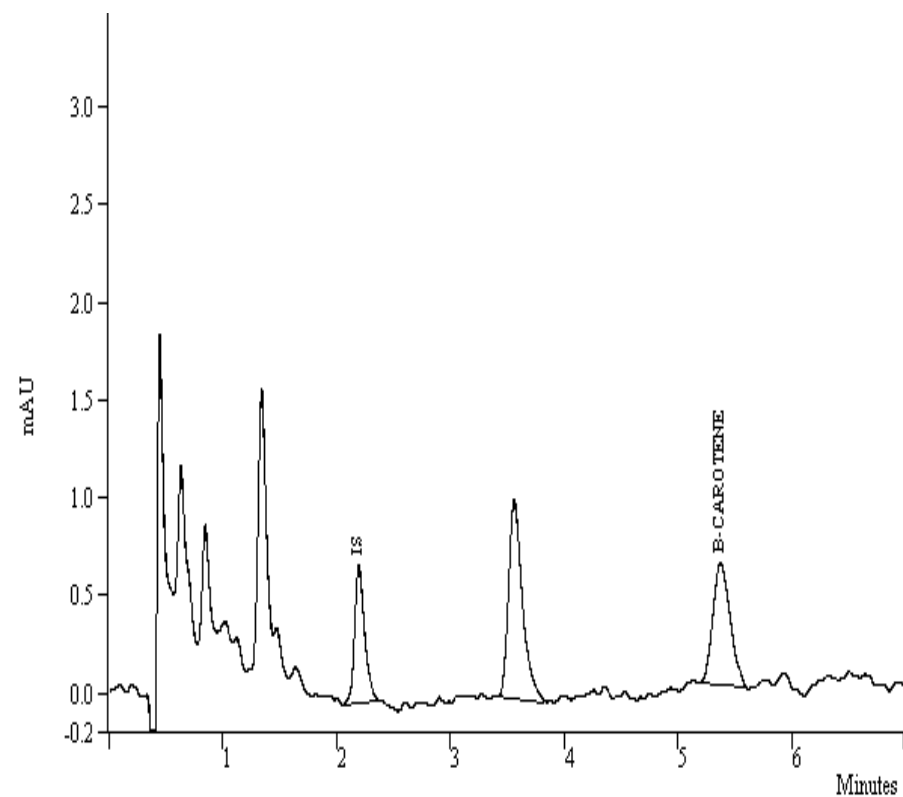


Fig. 2 :	Ei. of Plasma sample
	R.T. 2.1 Internal Standard
	R.T. 5.3 beta-Carotene 350 µg/L