

# METHEMOGLOBIN

Spectrophotometric determination  
of Methemoglobin on whole blood

25 tests

**REF** CM10-25T

## INTENDED USE

Kit for quantitative *in vitro* determination of Methemoglobin on whole blood.

## PRINCIPLE

Methemoglobin is a hemoglobin derivative with trivalent iron, which is formed by the oxidation of divalent iron. Methemoglobin loses its ability to deliver oxygen. Methemoglobin accumulation in erythrocytes can result from either congenital or acquired processes. Cases of acquired methemoglobin are frequent, due to intoxications caused by substances with direct oxidizing action, such as nitrites and nitrates or after some metabolic transformations in the organism, like some drugs (salicylic acid, pyramidon, sulphamide) or derivatives used in industry (aniline, toluol and benzol derivatives).

The method reads the absorbance of methemoglobin at 630 nm. Azide addition causes elimination of methemoglobin as it is almost completely transformed into azide methemoglobin. The reduction of absorbance at 630 nm after azide addition is proportional to the methemoglobin concentration.

## REAGENTS

Kit components

**REF** CM10-25T

### REAGENT 1

CM10-25TR1: 2 x 55 ml

Phosphate buffer pH 6.6

### REAGENT 2

CM10-25TR2: 1 x 6 ml

Sodium azide 70 mM

### REAGENT 3

CM10-25TR3: 1 x 3 ml

Potassium ferricyanide 50 g/L

(\* ) Dangerous reagents are marked by an asterisk. Refer to MSDS.

STABILITY: stored at 2-8°C, sealed reagents are stable up to the expiration date on the label.

## SAMPLE

Whole blood anticoagulant with heparin or EDTA.

STABILITY: at least 5 days at room temperature or at 2-8°C.

## MANUAL ASSAY PROCEDURE

Wavelength: 630 nm  
Optical path: 1 cm  
Reading: Reagent 1  
Temperature: room temperature (0-25°C)  
Method: spectrophotometric  
Reaction time: 10 minutes

## PREPARATION OF THE HEMOLYSATE

Pipette into a test tube:

Distilled water	3.9 ml
Sample	0.1 ml
Reagent 1	4.0 ml

## DETERMINATION OF METHEMOGLOBIN %

Pour 3.0 ml of hemolysate into two cuvettes labeled CUVETTE 1 and CUVETTE 2.

### CUVETTE 1

Read the absorbance (D1) directly at 630 nm against Reagent 1.

Add 0.1 ml of Reagent 2 and mix well.

Read the absorbance (D2) at 630 nm against Reagent 1.

### CUVETTE 2

Add 0.1 ml of Reagent 3, mix well and wait 2 minutes.

Read the absorbance (D3) at 630 nm against Reagent 1.

Add 0.1 ml of Reagent 2 and mix well.

Read the absorbance (D4) at 630 nm against Reagent 1.

## CALCULATION

Methemoglobin % = [(D1-D2) / (D3-D4)] x 100

## REFERENCE VAUES

Methemoglobin: in adults up to 1% of total hemoglobin; in children up to one year old up to 1.5% of total hemoglobin.  
Values over 1.5% of total hemoglobin are considered pathological.

## DISPOSAL

The product must be used for professional assay only. Dispose of the product according to national/international laws.

## WARNINGS AND PRECAUTIONS

REAGENT 1: Non dangerous.



### REAGENT 2

H412 Harmful to aquatic life with long lasting effects.

P273 Do not release into the environment.

STANDARD: Non dangerous.

## REFERENCE

Available on request.

## MANUFACTURER



FAR

Via Fermi, 12 - 37026 Pescantina - VERONA - ITALY

tel +39 045 6700870

website <http://www.farddiag.com>

e-mail: [order@farddiag.com](mailto:order@farddiag.com)

e-mail: [farddiag@farddiag.com](mailto:farddiag@farddiag.com)



## KEY SYMBOLS

	In Vitro diagnostic medical device
	batch number
	catalog number
	temperature limits
	use by
	caution
	read instructions for use

Ed. 01 - May 2022