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## Microscopy

### Amido black 10 B (C.I.20470)

for electrophoresis



In Vitro Diagnostic Medical Device



for staining of electrophoretically separated serum proteins

This staining dye "Amido black 10 B (C.I. 20470) - for electrophoresis" is used for human-medical cell diagnosis and serves the purpose of the investigation of sample material of human origin. It is a dry staining dye that is used to prepare a staining solution, that when used together with other in vitro diagnostic products from our portfolio makes target structures in specimen materials (by fixing, staining with the above amido black 10 B solution, decolorize, electrophoresis) evaluable for diagnostic purposes.

#### Principle

Proteins possess free amino and carboxyl groups and are charged positively or negatively in aqueous solutions, depending on the pH. In an alkaline milieu they are negatively charged and migrate in the electric field towards the anode at a speed that depends on the number of charges they carry. This difference in migration velocities makes it possible to separate the serum protein mixture into groups of individual components. The separated protein fractions are stained with amido black and measured photometrically or densitometrically.

As a rule, five fractions are observed after staining - albumin,  $\alpha_1$ - and  $\alpha_2$ -globulins,  $\beta_1$ - and  $\beta_2$ -globulins, and  $\gamma$ -globulins - where albumin has the highest migration velocity and  $\gamma$ -globulin the lowest.

Differentiation of serum proteins by electrophoresis is important for the diagnosis and prognosis of numerous diseases. Changes in protein composition can be found e.g. in inflammatory diseases, tumors, liver diseases, antibody deficiency syndromes or disturbances in protein balance.

#### Sample material

Serum, undiluted

#### Reagents

Cat. No. 101167

Amido black 10 B (C.I. 20470) 25 g, 100 g, 25 kg for electrophoresis

Color Index No.: 20470

Color Index Name: Naphthol blue black

#### Also required:

Cat. No. 100063	Acetic acid (glacial) 100% anhydrous for analysis EMSURE® ACS,ISO,Reag. Ph Eur	1 l, 2.5 l
Cat. No. 100983	Ethanol absolute for analysis EMSURE® ACS,ISO,Reag. Ph Eur	1 l, 2.5 l, 5 l
Cat. No. 102103	Calcium lactate for soil tests	250 g
Cat. No. 108383	Tris(hydroxymethyl)amino-methane GR for analysis buffer substance ACS,Reag. Ph Eur	100 g, 500 g, 1 kg, 2.5 kg
Cat. No. 109057	Hydrochloric acid c(HCl) = 1 mol/l (1N) Titripur®	1 l, 2.5 l, 4 l Titripac®, 5 l
Cat. No. 116802	Agarose (low electroendosmosis) for electrophoresis	25 g, 250 g
Cat. No. B0375	Barbital	Sigma
Cat. No. 71290	Sodium azide purum p.a., ≥99.0% (T)	Aldrich

#### Sample preparation

The sampling must be performed by qualified personnel.

All samples must be treated using state-of-the-art technology. All samples must be clearly labeled. Suitable instruments must be used for taking samples and their preparation. Follow the manufacturer's instructions for application / use.

#### Reagent preparation

##### Tris-barbiturate buffer solution pH 8.6

For preparation of approx. 1000 ml solution mix:

Barbital	22.4 g
Tris(hydroxymethyl)amino-methane	44.3 g
Calcium lactate	0.553 g
Sodium azide	0.65 g
Redistilled water	800 ml
dissolve	
with hydrochloric acid 1 N	adjust the pH to 8.6 with
Redistilled water	make up to 1 l

##### Agarose gel

For preparation mix:

Agarose for electrophoresis	0.3 g
Tris-barbiturate buffer solution pH 8.6	30 ml
Bring to the boil while stirring	
Pour 16 ml of the hot, clear solution onto a GelBond film (e.g. 94 x 84 mm in size) to obtain an approx. 2 mm thick layer of gel.	
Store the gel overnight in a wet chamber.	

##### Fixing solution

For preparation of approx. 100 ml solution mix:

Ethanol	60 ml
Acetic acid 100%	10 ml
Distilled water	30 ml
mix	

The solution must be prepared at least 15 min before use.

##### Decolorizing solution

For preparation of approx. 2.2 l solution mix:

Ethanol	1 l
Distilled water	1 l
Acetic acid 100%	222 ml
mix	

##### Staining solution

For preparation of approx. 250 ml solution mix:

Amido black 10 B (C.I.20470)	12 g
Decolorizing solution	250 ml
dissolve and filter	

The freshly prepared staining solution should be filtered before use.

#### Procedure

Electrophoresis of the gel is carried out according to the instructions for the electrophoresis system used.

**Dry the gel** on a sheet of filter paper with hot air at 80 °C in a drying cabinet for approx. 20 min. The gel must be completely dry.

**Fix the gel**, placed in a gel holder, for 15 min in the fixing solution. Remove the gel and dry with hot air at 80 °C. The gel must be completely dry.

**Stain the dried, cooled gel** in the staining solution for 4 min.

**Decolorize** in 3 consecutive baths with decolorizing solution. Decolorize the gel until the background is clear and colorless. Take off the excess solution from the gel surface using filter paper. Then dry the gel at 80 °C. If necessary, clean the back of the gel with a moist cloth.

## Densitometric evaluation

Scan the gel in the densitometer at a wavelength of 570 nm.

## Result

Normal values*	Total protein	60 - 80 g/l
	Albumin	50 - 60 %
	$\alpha_1$ -Globulins	5 - 7 %
	$\alpha_2$ -Globulins	6 - 10 %
	$\beta$ -Globulins	8 - 13 %
	$\gamma$ -Globulins	10 - 18 %

\*(acc. to Kienholz)

## Technical notes

The electrophoresis system and the densitometer used should meet the requirements of a medical diagnostic laboratory.

Serum protein electrophoresis is not a standardized method. Each laboratory should determine its own reference ranges.

The freshly prepared staining solution should be filtered before use.

## Diagnostics

Diagnoses are to be made only by authorized and trained personnel.

Valid nomenclatures must be used.

Further tests must be selected and implemented according to recognized methods.

Suitable controls should be conducted with each application in order to avoid an incorrect result.

## Storage

Store Amido black 10 B (C.I. 20470) - for electrophoresis at +5 °C to +30 °C.

## Shelf-life

Amido black 10 B (C.I. 20470) - for electrophoresis can be used until the stated expiry date.

After first opening of the bottle, the contents can be used up to the stated expiry date when stored at +5 °C to +30 °C.

The bottles must be kept tightly closed at all times.

## Additional instructions

### For professional use only.

In order to avoid errors, the application must be carried out by qualified personnel only.

National guidelines for work safety and quality assurance must be followed.

Microscopes equipped according to the standard must be used.

## Protection against infection

Effective measures must be taken to protect against infection in line with laboratory guidelines.

## Instructions for disposal

The package must be disposed of in accordance with the current disposal guidelines.

Used solutions and solutions that are past their shelf-life must be disposed of as special waste in accordance with local guidelines. Information on disposal can be obtained under the Quick Link "Hints for Disposal of Microscopy Products" at [www.microscopy-products.com](http://www.microscopy-products.com). Within the EU the currently applicable REGULATION (EC) No 1272/2008 on classification, labelling and packaging of substances and mixtures, amending and repealing Directives 67/548/EEC and 1999/45/EC, and amending Regulation (EC) No 1907/2006 applies.

## Auxiliary reagents

Cat. No.	100063	Acetic acid (glacial) 100% anhydrous for analysis EMSURE® ACS,ISO,Reag. Ph Eur	1 l, 2.5 l
Cat. No.	100983	Ethanol absolute for analysis EMSURE® ACS,ISO,Reag. Ph Eur	1 l, 2.5 l, 5 l
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Cat. No.	116802	Agarose (low electroendosmosis) for electrophoresis	25 g, 250 g
Cat. No.	B0375	Barbital	Sigma
Cat. No.	71290	Sodium azide purum p.a., ≥99.0% (T)	Aldrich

## Hazard classification

Cat. No. 101167

Please observe the hazard classification printed on the label and the information given in the safety data sheet.

The safety data sheet is available on the website and on request.

## Main components of the product

Cat. No. 101167

C.I. 20470

$C_{22}H_{14}N_6Na_2O_9S_2$

M = 616.50 g/mol

## Other IVD products

Cat. No.	101383	Wright's eosin methylene blue solution for microscopy	100 ml, 500 ml, 2.5 l
Cat. No.	109204	Giemsa's azur eosin methylene blue solution for microscopy	100 ml, 500 ml, 1 l, 2.5 l
Cat. No.	115927	Certistain® Ponceau S (C.I. 27195) for microscopy	25 g



Consult instructions for use



Manufacturer



Catalog number



Batch code



Caution, consult accompanying documents



Use by YYYY-MM-DD



Temperature limitation

Status: 2016-04-28

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[www.microscopy-products.com](http://www.microscopy-products.com)

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