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Elektrophorese GmbH

Instructions for

Colloidal Staining with AzurGel-K

for Gel format 10 x 10 x 0,1 cm

Cat No.: GF 10002

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- **Basics**

This staining procedure uses the colloidal properties of Coomassie™ Brilliant Blue G 250 dyes in the presence of inorganic acid and a high concentration of salt. Optimal staining of proteins on a clear background can be achieved with this kit.

- **References**

Neuhoff V, Arold N, Taube D, Ehrhardt W (1988) Electrophoresis *9*, 255 - 262
Neuhoff V, Stamm R, Eibl, H (1985) Electrophoresis *6*, 427 - 448

- **Advantages**

Higher sensitivity as customary Coomassie™ Brilliant Blue dyes
Protein staining through the whole cross-cut. (< 1 mm)
Waterclear background after destaining
Stable fixing after staining
Suitable for Tris/Glycine/SDS- and Tris/Tricine-gels
Fewer changes of solution
Solvent free destaining using D.I.-water

- **Specification**

Stainer A:	500 ml, contains (Ammonium sulfate and Phosphoric acid)
Stainer B :	125 ml, contains Coomassie™ Brilliant Blue G 250 which is enough for 25 gels, 10 x 10 cm
Storage:	room temperature
Shelf Life:	6 month, see package
Detection limit:	6 ng BSA (with 5 mm well widths and 1 mm gel diameter)
Duaration:	~ 12 h, 3 – 4 steps
Handling:	Wear eye protector and gloves while handling the gels and solutions.

- **General Guidelines**

- Shaker
Accomplish fixing and staining with a shaker by 50- 70 rounds per minute.

- Staining Containers
Only use clean, dense staining containers to avoid evaporation of methanol. Ensure that the container diameter and depth is sufficient to permit gel coverage with 100 ml of solution per gel. If possible, stain only one gel per staining dish, but not more than two gels per staining dish.

- **Tris/Glycine/SDS gels**

1. Staining

Before staining, prepare the following solution:

55 ml	D.I. water
20 ml	methanol
20 ml	Stainer A
5 ml	Stainer B **(shake well before use)**

Incubate the gel on a shaker in the prepared solution for several hours or overnight. After 3 hours, the bands are visible, but the complete staining of the gel cross-cut is finished after 8.5 hours. The background of a gel with a low concentration of acrylamide (< 10 % T) is often more stained, because the colloidal particles enter into the large polyacrylamide pores of these gels.

2. Washing/Destaining

Put the gel in clean staining bowl and fill up to 200 ml with D.I. water. Shake the gel until the background of the gel becomes waterclear. Optimal destaining is reached after 7 hours, nevertheless it is possible to leave the gel in the water for up to three days (ie. over the weekend) without the loss of protein band intensity. Obstinate background staining can be cleaned with 250 ml/l methanol. Long term storage (more than 3 days) can cause a decrease of the protein bands intensity.

3. Storage

If you want to store the gel for a long time in a solution, add 200g/l Ammonium sulfate to the solution. This process eliminates the colour compounds from passing over to the solution and the bands remain stained.

4. Drying

If the gel is to be kept for documentation or other reasons, usage of AnaFix gel drying kit is recommended. The gels are dried by evaporation on the lab bench without shrinking. When staining with AzurGel-K time of incubation with AnaRapid gel drying solution should not exceed 5 –15 minutes or the destaining of the protein bands may occur.

- **Tris/Tricine-Gels**

1. Fixing

Incubate the gel for 15 minutes in the following solution:

40 ml/l	D.I. water
50 ml/l	methanol
10 ml/l	acetic acid (concentrated)

2. Staining

Incubate the gel after fixing for 15 minutes in the following solution:

55 ml	D.I. water
20 ml	methanol
20 ml	Stainer A

Afterwards, add 5 ml Stainer B **(shake well before use)** and incubate the gel on a shaker for several hours or overnight in this solution. After 3 hours bands are visible, but the complete staining of the gel cross- cut is accomplished after 8.5 hours.

3. Washing/Destaining

Put the gel in a second, clean staining bowl and wash it with 200 ml D.I. water. Then apply the same procedures as described in the Tris/Glycine instruction.

- **Cleaning**

10 ml	HCl 32 %
90 ml	methanol

This solution can be stored and may be used several times.

- **Trouble shooting**

Problem	reason/cause	action taken
Staining solutions contains solid particles of stainer compound	Aggregation of colloidal particles	This is normal – no action required
Gel matrix completely stained blue	Not enough Stainer A used, staining compound therefore not in colloidal format	Destain gel in 25 % methanol, stain again if necessary
Proteins after 12 hours of staining show weak staining effect	Not enough Methanol or evaporated	Most of staining compound too strong in colloidal solution; staining time is extremely extended
Bands from different proteins with similar amounts show variations in staining effectiveness.	Different strongness in affinity of the stainer is an obviously known effect	For quantifications, always use calibrated amounts of proteins of interest