SIGMA®
ELECTROPHORESIS

DOUBLE STAINING KIT

February 1993 Technical Bulletin No. DSK-100

The Double Staining procedure is a highly sensitive staining technique for proteins in polyacrylamide gels. This technique includes two steps: 1. Staining with Brilliant Blue G Colloidal Suspension, followed by 2. Staining with Silver Staining Solution. The sensitivity using both is 10 times more than using Silver Stain alone. The detection level is as low as 10 ng.

ITEMS PROVIDED: Sufficient to stain up to 20 mini gels (10 x 8 cm) or 10 large gels (18 x 16 cm).

Store all components at room temperature unless otherwise indicated.

Item	Product No.
Component 1 - Silver Nitrate and Ammonium Nitrate	S-6028
Component 2 - Tungstosilicic Acid	T-4791
Component 3 - Formaldehyde Solution	F-5775
Component 4 - Sodium Carbonate	S-5903
Gel Incubation Concentrate	A-1814
Brilliant Blue G Colloidal Concentrate	B-2025
One Empty Bottle	———
Technical Bulletin	DSK-100

ITEMS REQUIRED BUT NOT PROVIDED:

Fixing Solution	F-7264
Methanol	M-3641
Glacial Acetic Acid	A-6283
Ultra Pure Water (18 megohm-cm resistivity)	———

TECHNICAL TIPS:

1. Clean all containers with 50% (v/v) nitric acid and rinse thoroughly with ultra pure water.
2. Wear gloves during each step.
3. COMPLETELY submerge the gel in each solution.
4. Prepare all reagents and stock solutions using ultra pure water to obtain clear results.
5. A negative staining result may be obtained if samples (proteins or nucleic acids) are overloaded. If this occurs, the staining procedure should be repeated.

PREPARATION OF STOCK SOLUTIONS:

Prepare all stock solutions using ultra pure water.

1. **COMPONENT 1** - Product No. S-6028

   Add 98 ml of water to the bottle labeled "Component 1". Stir with a clear Teflon-coated stirring bar for approximately 15 minutes or until powder is completely dissolved.

2. **COMPONENT 2** - Product No. T-4791

   Add 85 ml of water to the bottle labeled "Component 2". Stir with a clean Teflon-coated stirring bar for approximately 15 minutes or until material is completely dissolved. Remove stirring bar, QS to 100 ml and invert bottles several times to obtain a homogeneous solution.

3. **COMPONENT 3** - Product No. F-5775

   Dilute the contents of the bottle labeled "Component 3" with 95 ml of water. Stir with a clean Teflon-coated stirring bar for approximately 15 minutes. Remove stirring bar, QS to 100 ml, and invert bottle several times to obtain a homogeneous solution.

4. **COMPONENT 4** - Product No. S-5903

   Add 900 ml of water to the empty bottle provided. Place a clean Teflon-coated stirring bar into the bottle. While stirring, slowly add the contents of the plastic bag labeled "Component 4". Material will quickly dissolve. Remove stirring bar, QS to 1000 ml, and invert bottle several times. Store at 4°C.

   Note:If water is added to the powder, a longer period of time is required for the material to dissolve.

5. **Brilliant Blue G Colloidal Concentrate** - Product No. B-2025

   Add 800 ml of deionized water to the bottle labeled Brilliant Blue G Colloidal Concentrate. Replace the cap and tighten. Mix by inversion. This 1X working solution should **NOT** be filtered. Store at 0-5°C once diluted.

   NOTE:If Brilliant Blue G Colloidal 1X Working Solution is not freshly prepared, mix by inversion prior to combining with methanol. After combining with methanol, the suspension is stable for only 4 hours.

PREPARATION OF REAGENTS:

Prepare all reagents using ultra pure water.

1. Gel Incubation Solution - Product No. A-1814
   Add 700 ml of water to the bottle labeled "Gel Incubation Solution". Stir with a clean Teflon-coated stirring bar until material is completely dissolved. Remove stirring bar, and QS to 1000 ml. Invert bottle several times to obtain a homogeneous solution.

2. Brilliant Blue G Colloidal Suspension
   IMMEDIATELY BEFORE USE, prepare by combining:

Brilliant Blue G Colloidal Stain, 1X Working Solution	160 ml
Methanol	40 ml

3. Rinsing Solution (25% methanol solution)
   Prepare by combining:

Methanol	50 ml
Ultra pure water	150 ml

4. Destaining Solution (25% methanol, 10% glacial acetic acid)
   Prepare by combining:

Methanol	125 ml
Glacial Acetic Acid	50 ml

   Dilute to 500 ml with ultra pure water

   NOTE: Always add acid to water.

5. Silver Staining and Development Solution
   PREPARE IMMEDIATELY BEFORE USE.
   Add the following components sequentially with stirring to a 150 ml beaker:

Water	35 ml
Prepared "Component 1"	5.0 ml
Prepared "Component 2"	5.0 ml
Prepared "Component 3"	5.0 ml
Prepared "Component 4"	50 ml

   Final Volume: 100 ml

   NOTE: For large gels prepare 3X the amount (300 ml total volume).

6. Stop Solution (5% Acetic Acid)
   Prepare solution by combining:

Water	190 ml
Glacial Acetic Acid	10 ml

   NOTE: Always add acid to water.

PROTOCOL FOR DOUBLE STAINING GELS AFTER ELECTROPHORESIS

PART A

Step 1: Fixing

Fix gels(s) with gentle agitation for 1 hour in 200 ml of Fixing Solution, (Prod. No. F-7264). (Alternatively, a 12% trichloroacetic acid solution may be used as the fixative.)

Step 2: Gel Equilibration

Replace the Fixing Solution from Step 1 with 200 ml of reconstituted Gel Incubation Solution (Prod. No. A-1814) and allow gel(s) to incubate for 1 hour with gentle agitation.

Step 3: Staining with Brilliant Blue G Colloidal Suspension

Replace the Gel Incubation Solution from Step 2 with 200 ml Brilliant Blue G Colloidal Suspension and allow gel(s) to incubate for 1 hour.

Step 4: Destaining

Rinse the gel in Rinsing Solution for 60 seconds with agitation. Destain the gel with Destaining Solution for 60 seconds with shaking. Replace with fresh Destaining Solution and allow to remain in the destain until ready to silver stain. If any precipitated dye remains on the surface of the gel when the gel is removed from the Destain Solution, gently wipe with a clean cotton ball which has been soaked in Rinsing Solution.

PART B

Step 5: Washing

Wash the gel from Step 4 twice in ultra pure water with gentle agitation (approx. 10 minutes for each wash).

Step 6: Silver Staining and Development

Transfer the gel from Step 5 into the Staining and Development Solution and incubate with gentle agitation for 20-30 minutes or until the desired band intensity is obtained. Refer to Table A for suggested staining times based upon size and thickness of the gels.

Step 7: Stopping

After the desired intensity is obtained, stop the gel staining reaction by transferring the gel into the Stop Solution. Incubate with gentle agitation for 20 minutes then transfer the gel(s) into water. The gel(s) may then be dried for storage.

Table A: Recommended gel treatment conditions.

	(Volume)		(Time)	
Step	Mini gel **(10 x 8 cm)**	Large gel **(18 x 16 cm)**	Thickness **(0.5-1.0 mm)**	Thickness **(1.5-3.0 mm)**
1. Fixing	200 ml	400 ml	1 hour	1 hour
2. Gel Equilibration	200 ml	200 ml	1 hour	1 hour
3. Brilliant Blue G Staining	200 ml	400 ml	1 hour	1 hour
4. Destaining	200 ml	400 ml	1/2-1 hour	1/2-1 hour
5. Washing	200 ml	400 ml	2 x 10 min	2 x 20 min
6. Staining & Developing	100 ml	300 ml	20-30 min	30-40 min
7. Stopping	200 ml	400 ml	10 min	20 min

SIGMA®
CHEMICAL COMPANY
P.O. BOX 14508, ST. LOUIS, MO 63178 USA

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SIGMA-ALDRICH s.r.o. • Křižíkova 27 • 180 00 Praha 8 • Česká republika • Tel.: 02 2366973 • Fax: 02 2364141
SIGMA-ALDRICH CORP., Unit No. 111, Sunny Place, 3-6-728, Opp. SBH., Himayathnagar, Hyderabad 500 029 (AP), India • Tel.: 91 (842) 661127 • Fax: 91 (842) 661127
NEW DELHI, B-4/158, Safdarjung Enclave, New Delhi-110 029 • Tel.: 91 (11) 688-6872 • Fax: 91 (11) 688-6873

Printed in the USA