Shaded Areas indicate that text has been modified, added or deleted.

**TITAN GEL Silver Stain Kit**

**Ingredients:** Each vial contains dithiothreitol and fillers.

**PREPARATION FOR USE:** Dilute the vial of silver reducing agent in 2 L of deionized water.

**Recipes:**

1. **TITAN GEL Silver Stain Fixative**

   **Ingredients:**
   - Silicotungstic acid
   - Deionized water.

   **Storage and Stability:** The packaged reagent should be stored at room temperature (15 to 30°C) in the dark and is stable until the expiration date on the vial. The dissolved reagent must be stored at 2 to 6°C. Both are stable until the expiration date on the vial.

   **Reagent IIA**

   **Ingredients:**
   - Silicotungstic acid
   - Deionized water.

   **Storage and Stability:** IRRITANT - NEVER PIPETTE BY MOUTH.

   **Reagent IIB**

   **Ingredients:**
   - Silicotungstic acid
   - Deionized water.

   **Storage and Stability:** IRRITANT - NEVER PIPETTE BY MOUTH.

   **REAGENT I**

   **Ingredients:**
   - Silicotungstic acid
   - Deionized water.

   **Storage and Stability:** IRRITANT - NEVER PIPETTE BY MOUTH.

   **REAGENT II**

   **Ingredients:**
   - Silicotungstic acid
   - Deionized water.

   **Storage and Stability:** IRRITANT - NEVER PIPETTE BY MOUTH.

   **REAGENT III**

   **Ingredients:**
   - Silicotungstic acid
   - Deionized water.

   **Storage and Stability:** IRRITANT - NEVER PIPETTE BY MOUTH.

   **REAGENT IV**

   **Ingredients:**
   - Silicotungstic acid
   - Deionized water.

   **Storage and Stability:** IRRITANT - NEVER PIPETTE BY MOUTH.

   **REAGENT V**

   **Ingredients:**
   - Silicotungstic acid
   - Deionized water.

   **Storage and Stability:** IRRITANT - NEVER PIPETTE BY MOUTH.

   **REAGENT VI**

   **Ingredients:**
   - Silicotungstic acid
   - Deionized water.

   **Storage and Stability:** IRRITANT - NEVER PIPETTE BY MOUTH.
While the gel is in the Reducing Agent, prepare the Working Silver Stain Reagent I. Place 0.7 mL of each of Reagents IIA, IIB and IIC in a sterile cup.

**Materials needed but not provided:**
- Methanol
- Glacial Acetic Acid
- Glass Shaker
- Staining Dishes
- Forceps

**STAINING SOLUTION:**

1. Reconstitute one vial each of Reagent IIA, IIB and IIC with 7 mL deionized water.
2. Add 0.7 mL of Reagent IID and stir vigorously.
3. Stir vigorously. The solution should be clear. If the solution is not clear, discard it since it may cause precipitation. If the solution is cloudy, dilute with 10 mL of deionized water.

**Application:**

1. Pour approximately 125 mL of working Silver Stain into a staining dish.
2. Pour an equal volume of working Silver Stain on top of the gel. Do not allow the gel to remain in the Staining Chamber for more than 10 minutes.
3. The gel should be washed with 50 mL of deionized water.
4. The gel should be allowed to dry for 10 minutes before staining.
5. To stop the staining process, add 25 mL of stop-bath solution.

**Materials Needed but not Provided:**
- Glass Shaker
- Transparent Film
- TITAN GEL Silver Stain Buffer
- Forceps
- Sterile Cup
- TITAN GEL Silver Stain Reagent I
- TITAN GEL Silver Stain Reagent II
- TITAN GEL Silver Stain Reagent IID
- TITAN GEL Silver Stain Reagent IIB
- TITAN GEL Silver Stain Reagent IIA
- TITAN GEL Silver Stain Reagent IIB
- TITAN GEL Silver Stain Reagent IIA
- TITAN GEL Silver Stain Tissue Paper
- TITAN GEL Silver Stain Buffer
- TITAN GEL Silver Stain Reagent B
- TITAN GEL Silver Stain Reagent A
- TITAN GEL Silver Stain Reagent C
- TITAN GEL Silver Stain Reagent D
breathe. Keep away from heat, sparks or open flame.

Preparation for Use: Reaction ID should be ready as soon as prepared. Storage and Stability: Reagent ID should be stored at room temperature (15 to 30°C) and is stable until the expiration date on the vial.

*Signs of Deterioration: Reaction ID should be a homogeneous solution free of precipitates.

9. TITAN GEL Silver Stain Gel

**Ingredients:** Each gel contains agarose in barbiturate buffer with 0.1% sodium azide added as a preservative.

**WARNING:** FOR IN-VITRO DIAGNOSTIC USE ONLY. See reagent package insert for complete precautions.

**Preparation for Use:** The gels are ready to use as packaged.

**Storage and Stability:** The gel should be stored at room temperature (15 to 30°C) and is stable until the expiration date indicated on the package. The gels must be stored in the protective packaging in which they are shipped. DO NOT REFRIGERATE OR FREEZE THE GELS.

**Signs of Deterioration:** Any of the following conditions may indicate deterioration of the gel: (1) crystalline appearance indicating the agarose has been frozen, (2) cracking and peeling indicating drying of the agarose, (3) bacterial growth indicating contamination.

10. TITAN GEL Silver Stain Buffer

**Ingredients:** The buffer contains barbiturate, sodium barbiturate, calcium lactate and 0.1% sodium azide added as a preservative; pH 8.4-8.8.

**WARNING:** FOR IN-VITRO DIAGNOSTIC USE ONLY. DO NOT INGEST. CAUTION: The buffer contains barbiturate, which, in sufficient quantity can be toxic. Refer to sodium azide precaution.

**Preparation for Use:** Dissolve package of buffer in 1500 mL deionized water. The buffer is ready for use when all material is completely dissolved.

**Storage and Stability:** The packaged buffer should be stored at 15 to 30°C until the expiration date indicated on the package. Diluted buffer is stable six months stored at 15 to 30°C.

**Signs of Deterioration:** Discard packaged buffer if the material shows signs of dampness or decolorization. Dissolve diluted buffer if it becomes turbid.

**SODIUM AZIDE WARNING**

To prevent the formation of toxic vapors, sodium azide should not be mixed with acidic solutions. When dissolving reagents containing sodium azide, always flush with copious quantities of water to prevent the formation of metallic azides, which, when highly concentrated in metal plumbing, are potentially explosive. In addition to purging pipes with water, plumbing should occasionally be decontaminated with 10% NaOH.

**SPECIMEN COLLECTION AND HANDLING**

**Specimen:** The specimen may be urine or cerebrospinal fluid. Other body fluids may be used. When using plasma, ensure the specimen is free of fibrinogen which obscures the beta-gamma zone.

**Preparation for Use:** CSF and urine samples should not be concentrated, but rather applied directly to the gel. Other body fluids should be diluted or concentrated such that the total protein is at least 10% of the mixture and placed in a dish containing 500 mL deionized water. Place the sample on the gel and allow it to dry or place it in a forced air oven at 70°C for 5-10 minutes until dry. Visually inspect the gel for the presence of the protein bands. If scanning is desired, a white light (no filter) should be used on a densitometer or spectrophotometer.

**Stability of End Product:** The protein bands identified with TITAN GEL Silver Stain Gel is storable for an indefinite period of time when kept in the dark.

**Quality Control:** TITAN GEL High Resolution Protein Marker (Cat. No. 5141) is recommended as a quality control. It should be diluted 1:10 or 1:5 with saline or buffer, depending on desired intensity, before applying to the agarose.

**RESULTS**

Refer to the migration patterns of several proteins which may be identified using the TITAN GEL Silver Stain Procedure. Additional bands may be present in abnormal cerebrospinal fluid samples.

Figure 1: TITAN GEL Silver Stain

**PERFORMANCE CHARACTERISTICS**

The agarse silver stain calibration curve has a typical “S” shape. Non-linearly is observed over 2 mg/ml protein concentration. The intensity of the bands varies with the concentration of protein present per band, and (b) on the time allowed for development. TITAN GEL Silver Stain has sensitivity of 0.6 ng/ml (60 pg/cmband). The linear range (darkest band) (albumin) was determined to be 1.5 U.O. at a total protein concentration of 0.06 ug before electrophoresis.
Each vial contains dithiothreitol and fillers.

PREPARATION FOR USE:

**WARNING:** FOR IN-VITRO DIAGNOSTIC USE ONLY.

**SUMMARY**

High resolution electrophoresis attempts to achieve better resolution of the proteins beyond the classical five band patterns and thus increase the diagnostic usefulness of the protein patterns. Silver stain further increases the diagnostic usefulness of electrophoresis by increasing the sensitivity of the system.

Silver staining has been found to be up to 100 times more sensitive than traditional methods for staining protein bands in polyacrylamide gels. Silver stain further increases the diagnostic usefulness of electrophoresis by increasing the sensitivity of the system.

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Kerenyi and Gallaya first published silver stain used to detect electrophoretically separated proteins. Merrit et al. developed a silver stain procedure that was compatible with polyacrylamide gels and was not subject to the limitations of the Kerenyi and Gallaya procedure. The TITAN GEL Silver Stain Kit eliminates the historical argentation problems such as reproducibility, background staining, gel thicknesses and the presence of reducing groups in the gel formulations.

**PRINCIPLE**

Silver ions are believed to complex with various groups on protein molecules. Illumination of the reaction (photo-catalysis) will increase the sensitivity of the reaction on the agarose gels. Typical nucleation sites are formed for the deposition of silver with eventual reduction to metallic silver, if alkaline conditions prevail.

Following electrophoresis, the separated proteins must be processed through the following steps:

**Fixation:** Glutaraldehyde covalently attaches to the protein allowing for the reduction of silver ions to metallic silver and its resultant deposition.

**Acceleration:** The Accelerator reagent reduces the surface tension of the protein samples, allowing for the diffusion of silver ions into the gels.

**Reduction:** The dithiothreitol is added to fully reduce the protein. This increases the number of silver sites and enhances the sensitivity of the system.

Silver staining is made up at an alkaline pH to provide the necessary medium for silver precipitation.

**Stop- Bath:** Acetic acid is used to stop the staining reaction.

REAGENTS

**1. TITAN GEL Silver Stain Fixative**

Ingredients: Each vial contains silver nitrate.

Stain Procedure must be ordered individually.

**2. TITAN GEL Silver Stain Accelerator**

Ingredients: Each vial contains ammonium nitrate.

Stain Procedure must be ordered individually.

**3. TITAN GEL Silver Stain Reducing Agent**

Ingredients: Each vial contains dithiothreitol and fillers.

Stain Procedure must be ordered individually.