bio-techne®
R&D SYSTEMS

CometAssay® Silver Staining Components Kit

Catalog Number 4254-200-K

200 samples

This package insert must be read in its entirety before using this product.
For research use only. Not for use in diagnostic procedures.

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INTRODUCTION

CometAssay® Silver Staining Component Kit is designed for the convenient silver staining of comet assay or single cell gel electrophoresis results. Using the CometAssay Silver Staining Component Kit, permanent records that can be visualized using standard light microscopy are prepared, thereby avoiding the problems associated with fluorescent stains and epifluorescence microscopy.

PRINCIPLE OF THE ASSAY

The CometAssay Silver Staining Components Kit were designed specifically for use with the CometAssay® system and CometSlides™ to minimize unwanted background and the amount of hazardous waste generated by silver nitrate.

LIMITATIONS OF THE PROCEDURE

- FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
- Do not mix or substitute reagents with those from other lots or sources.
- Variations in sample collection, processing, and storage may cause sample value differences.

TECHNICAL HINTS

- When mixing or reconstituting protein solutions, always avoid foaming.
- To avoid cross-contamination, change pipette tips between additions of each sample and reagent. Also, use separate reservoirs for each reagent.

PRECAUTIONS

The acute and chronic effects of overexposure to reagents of this kit are unknown. Safe laboratory procedures should be followed, and protective clothing should be worn when handling kit reagents.

This kit contains small quantities of hazardous materials: 2.8% Formaldehyde is found in 20X Staining Reagent #2 (R&D Systems, Catalog # 4254-200-02) and 10% tungstosilicic acid is found in 20X Staining Reagent #3 (R&D Systems, Catalog # 4254-200-03). Consult the SDS sheets for details.

The physical, chemical, and toxicological properties of the products contained within this kit may not yet have been fully investigated. The use of gloves, lab coats, and eye protection while using any chemical reagents is recommended.

The final Staining Solution is considered hazardous material. Disposal should be performed per local and state regulations. It is recommended to tap solution off the slide into a container for safe disposal.

MATERIALS PROVIDED & STORAGE CONDITIONS

Do not use past kit expiration date.

PART	PART #	AMOUNT PROVIDED	STORAGE OF UNOPENED MATERIAL
20X Staining Reagent #1	4254-200-01	1.2 mL	Room temperature
20X Staining Reagent #2	4254-200-02	1.2 mL	
20X Staining Reagent #3	4254-200-03	1.2 mL	
2X Staining Reagent #4	4254-200-04	1.2 g	
10X Fixation Additive	4254-200-05	2.2 mL	

OTHER MATERIALS REQUIRED

- CometAssay Kit (R&D Systems®, Catalog # 4250-050-K)
- Pipettors and pipette tips
- Methanol
- Glacial Acetic Acid
- Deionized water
- 80% Ethanol

REAGENT PREPARATION

Fixation Solution - Prepare immediately before fixation (50 µL per sample). Mix per sample:

Component	Volume
10X Fixation Additive	10 µL
Distilled water	30 µL
Methanol	50 µL
Glacial Acetic Acid	10 µL

2X Staining Reagent #4 - Before first use, add 12 mL of distilled water to bottle, stir until dissolved and store at 2-8 °C. Stable for 3 months after suspension. **Before each use, warm to room temperature.**

Staining Solution - Prepare the Staining Solution (50 µL/sample) immediately before staining following the instructions below. After adding the reagents, mix by tapping the tube.

Component	1 sample	10 samples
Distilled water	35 µL	350 µL
20X Staining Reagent #1	5 µL	50 µL
20X Staining Reagent #2	5 µL	50 µL
20X Staining Reagent #3	5 µL	50 µL
2X Staining Reagent #4	50 µL	500 µL

Stop Solution - Prepare a 5% Glacial Acetic Acid solution. 100 µL per sample area is required.

ASSAY PROTOCOL

This protocol describes using the kit with CometSlides, but it can be adapted for use with other slides. To reduce assay-to-assay variability, slides should be dried, fixed, and then silver stained.

DRYING

After performing the CometAssay or other single cell electrophoresis assay, slides should be dried completely and then fixed. To accelerate the drying step, immerse the slides into cold 80% Ethanol for 5 minutes, gently tap off excess Ethanol and air dry at room temperature or on a slide dryer using a maximum of 45 °C temperature.

FIXATION AND SILVER STAINING

Fixation is recommended for reproducible staining between assays. After electrophoresis and drying, samples are covered in fixation solution.

1. Cover the sample area with 100 µL of Fixation Solution.
2. Incubate for 20 minutes at room temperature.
3. Rinse in distilled water for 30 minutes. Removal of all residual acetic acid is essential.
4. Cover sample area with 100 µL of Staining Solution.
5. Incubate at room temperature for 5-20 minutes. Intensity of staining can be visualized under the microscope using a 10X objective, and reaction stopped when comets are easily visible.
6. Stop reaction by covering samples with 100 µL Stop Solution and incubate for 15 minutes.
7. Rinse in distilled water.
8. Air dry and store **in the dark**.

REFERENCES

1. Black, J.A. (1985) Electrophoresis. **6**:27.
2. Delincee, H. (1997) Newsletter (6). Kinetic Imaging Inc. Liverpool, UK.
3. Gottlieb, M. and M. Chavko (1987) Anal. Biochem. **165**:33.