

TREVIGEN[®] Instructions

For Research Use Only. Not For Use In Diagnostic Procedures

Silver Staining Components (CometAssay[®] Silver Staining Kit)

200 Samples

Catalog # 4254-200-K

Silver Staining Components **(CometAssay[®] Silver Staining Kit)**

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I. Background

Trevigen's **Silver Staining Components** are designed for the convenient silver staining of CometAssay[®] or single cell gel electrophoresis results. Using the **CometAssay[®] Silver Staining Kit**, permanent records that can be visualized using standard light microscopy are prepared, thereby avoiding the problems associated with fluorescent stains and epifluorescence microscopy.

The Silver Staining kit was designed specifically for use with CometSlides[™] to minimize unwanted background and the amount of hazardous waste generated by silver nitrate.

II. Precautions and Limitations

1. For Research Use Only. Not for use in diagnostic procedures.
2. The Silver Staining kit contains small quantities of hazardous materials: 2.8% Formaldehyde is found in Cat# 4254-200-02 and 10% tungstosilicic acid is found in Cat# 4254-200-03. Please consult the MSDS sheets for details. The physical, chemical, and toxicological properties of the products contained within this kit may not yet have been fully investigated. Trevigen recommends the use of gloves, lab coats, and eye protection while using any chemical reagents. Trevigen assumes no liability for damages resulting from handling or contact with these products.

III. Materials Supplied

This protocol describes using the kit with CometSlides[™], but it can be adapted for use with other slides.

<u>Silver Staining Components</u>	<u>Catalog #</u>	<u>Amount</u>	<u>Storage</u>
20X Staining Reagent #1	4254-200-01	1.2 ml	Room temp.
20X Staining Reagent #2	4254-200-02	1.2 ml	Room temp.
20X Staining Reagent #3	4254-200-03	1.2 ml	Room temp.
*2X Staining Reagent #4	4254-200-04	1.2 g	Room temp.
10X Fixation Additive	4254-200-05	2.2 ml	Room temp.

*Suspend in 12 ml dH₂O and store at 4°C. Stable for 3 months after suspension.

IV. Materials/Equipment Required But Not Supplied

1. CometAssay[®] Kit (please see page 4)
2. Pipettors and pipette tips
3. Methanol
4. Glacial Acetic Acid
5. High quality deionized water (dH₂O).

V. Reagent Preparation

1. Fixation solution

Prepare immediately before fixation. Mix per sample:

10X Fixation Additive (Cat# 4254-200-05)	10 µl
dH ₂ O	30 µl
methanol	50 µl
glacial acetic acid	10 µl

2. 2X Staining Reagent #4 (Cat# 4254-200-04)

Before first use, add 12 ml of dH₂O to bottle, stir until dissolved and store at 4°C. **Before each use, warm to room temperature.**

3. Staining solution (prepare immediately before staining)

The staining reagents 1, 2 and 3 are ready to use in the staining solution as described here.

Per sample, mix in a microtube:

dH ₂ O	35 µl
20X Staining Reagent #1 (Cat# 4254-200-01)	5 µl
20X Staining Reagent #2 (Cat# 4254-200-02)	5 µl
20X Staining Reagent #3 (Cat# 4254-200-03)	5 µl

Mix by tapping tube and add 50 µl 2X Staining Reagent #4.

For 10 samples:

dH ₂ O	350 µl
20X Staining Reagent #1 (Cat# 4254-200-01)	50 µl
20X Staining Reagent #2 (Cat# 4254-200-02)	50 µl
20X Staining Reagent #3 (Cat# 4254-200-03)	50 µl

Mix by tapping tube and add 500 µl 2X Staining Reagent #4.

4. Stop solution

Prepare a 5% acetic acid solution. 100 µl per sample area is required.

VI. Assay Protocol

To reduce assay-to-assay variability, slides are dried, fixed and then silver stained.

Drying

Slides should be dried completely before the fixation step. To accelerate the drying step, simply immerse the slides into cold 80% ethanol for 5 minutes, gently tap off excess and air dry at room temperature or at a maximum of 45°C.

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 μ l of Fixation solution prepared in section V: *Reagent Preparation*.
2. Incubate for 20 minutes at room temperature.
3. Rinse in dH₂O for 30 minutes. (Removal of all residual acetic acid is essential.)
4. Cover sample area with 100 μ l of Staining Solution prepared in section V: *Reagent Preparation*.
5. Incubate at room temperature for 5 to 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 μ l 5% acetic acid and incubate for 15 minutes.
7. Rinse in dH₂O.
8. Air dry and store in the dark.

VII. Warning/Safety

The final Silver Staining solution (prepared in section V, step 3) 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.

VIII. References

1. Black J.A. 1985. A silver stain for isoelectric focusing in agarose gel and its application for analyzing unconcentrated cerebrospinal fluid *Electrophoresis* **6**: 27-29.
2. Delincee H. 1997. Silver staining of DNA in the "comet assay". *Comet Newsletter* (6). Kinetic Imaging Inc. Liverpool, UK
3. Gottlieb, M. and M. Chavko. 1987. Silver staining of native and denatured eucaryotic DNA in agarose gels. *Anal. Biochem.* **165**: 33-37.

IX. Related Products Available from Trevigen

CometAssay® Kits:

Catalog #	Description	Size
4250-050-ESK	CometAssay® Starter Kit	each
4250-050-ES	CometAssay® ES	each
4250-050-K	CometAssay® Kit (25 x 2 well slides)	50 samples
4251-050-K	CometAssay® Silver Kit	50 samples
4252-040-K	CometAssay® HT (2 x 20 well slides)	40 samples
4253-096-K	CometAssay® Kit (96 well, 1 slide)	96 samples

FLARE™ Assay Kits:

Catalog #	Description	Damage Recognized	Size
4040-100-FK	Fpg Kit	8-oxoguanine, DNA containing formamidopyrimidine moieties	75 samples
4130-100-FK	hOGG1 Kit	8-oxoguanine, DNA containing formamidopyrimidine moieties	75 samples
4045-01K-FK	Endonuclease III Kit	Thymine Glycol, 5,6-dihydro-thymine, urea, 5-hydroxy-6-hydrothymine, 5,6-dihydro-uracil, alloxan, 5-hydroxy-6-hydrouracil, uracil glycol, 5-hydroxy-5-methylhydantoin, 5-hydroxycytosine, 5-hydroxy-uracil, methyltartonylurea, thymine ring saturated or fragmentation product	75 samples

Accessories:

Catalog #	Description	Size
3950-300-02	FLARE™ Slides (3 well)	100 slides
4250-050-03	CometSlide™ (2 well)	25 slides
4252-200-01	CometAssay® HT Slide (20 well)	10 slides
4253-960-03	96 Well CometSlide™	10 slides
4256-010-CC	Alkaline CometAssay® Control Cells	1 set
4257-010-NC	Neutral CometAssay® Control Cells	1 set
4380-096-K	HT 8-oxo-dG ELISA Kit II	96 wells
4870-500	10X PBS, Ca ⁺⁺ and Mg ⁺⁺ free	500 ml

PARP Assay Kits:

Catalog #	Description	Size
4520-096-K	HT PARP in vivo Pharmacodynamic Assay II	96 tests
4676-096-K	HT Universal Chemiluminescent PARP Assay	96 tests
4677-096-K	HT Universal Colorimetric PARP Assay	96 tests
4684-096-K	HT Colorimetric PARP/Apoptosis Assay	96 tests
4685-096-K	HT Chemiluminescent PARP /Apoptosis Assay	96 tests
4690-096-K	HT Homogeneous PARP Inhibition Assay	96 tests

The product accompanying this document is intended for research use only and is not intended for diagnostic purposes or for use in humans.

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