

CometAssay[®] Silver Staining Kit

200 Samples

Catalog # 4254-200-K

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I. Background

Trevigen's **CometAssay[®] Silver Staining Kit** is 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 micro-scropy 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>Component</u>	<u>Amount</u>	<u>Storage</u>	<u>Catalog #</u>
10X Fixation Additive	2.2 ml	RT	4254-200-05
20X Staining Reagent #1	1.2 ml	RT	4254-200-01
20X Staining Reagent #2	1.2 ml	RT	4254-200-02
20X Staining Reagent #3	1.2 ml	RT	4254-200-03
*2X Staining Reagent #4	1.2 g	RT	4254-200-04

*To be resuspended in 12 ml dH₂O and stored at 4 °C, stable for 3 months after resuspension.

**Other component stable for 1 year.

IV. Materials/Equipment Required But Not Supplied

1. CometAssay[®] kit (please see page 3)
2. Pipettors and pipette tips
3. Methanol
4. Glacial acetic acid
5. High quality deionized water (dH₂O). For best results, use 18 mΩ dH₂O for all steps requiring water.

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. Store at 4 °C, prewarm to room temperature before each use.

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, then add:	
2X Staining Reagent #4 (Cat# 4254-200-04)	50 µl
(at room temperature) from item 2 above.	

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, then add:	
2X Staining Reagent #4 (Cat# 4254-200-04)	500 µl
(at room temperature) from item 2 above.	

4. Stop solution

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

VI. Assay Protocol

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

A. 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 RT or at max. 45°C.

B. Fixing

Fixation is recommended as it improves repeatability of staining between assays. After electrophoresis and drying, samples are covered in fixation solution.

- a. Cover the sample area with 100 µl of Fixation solution (refer to section V, item 1, *Reagent Preparation*).
- b. Incubate for 20 minutes at room temperature.
- c. Rinse in dH₂O for 30 minutes.

C. Staining reaction

- a. Cover sample area with 100 µl of Staining Solution prepared in section V, item 3.
- b. Incubate at room temperature for 5 to 20 minutes. (Intensity of staining can be visualized under the microscope using a 10X objective and the reaction stopped when comets are easily visible).
- c. Stop reaction by covering samples with 100 µl 5% acetic acid and incubate for 15 minutes.
- d. Rinse in deionized water.
- e. Air dry.
- f. Store in the dark.

VII. References

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.

Delincee H. 1997. Silver staining of DNA in the "comet assay". Comet Newsletter (6). Kinetic Imaging Inc. Liverpool, UK

Gottlieb, M. and M. Chavko. 1987. Silver staining of native and denatured eucaryotic DNA in agarose gels. Anal. Biochem. **165**: 33-37.

VIII. Related Products Available from Trevigen

CometAssay[®] Kits:

Catalog #	Description	Size
4250-050-ES	CometAssay [®] Electrophoresis System (ES)	each
4250-050-ESK	CometAssay [®] ES Starter Kit	each
4250-050-K	CometAssay [®] Kit	50 samples
4252-040-K	CometAssay [®] HT	40 samples
4251-050-K	CometAssay [®] Silver Kit	50 samples
4253-096-K	CometAssay [®] Kit 96 Wells	96 samples
4252-040-K	CometAssay [®] Higher Throughput Kit	40 samples

DNA Damage Antibodies:

Catalog #	Description	Size
4411-PC-100	γ-H2AX polyclonal	100 µl
4410-PC-100	Fen-1 polyclonal	100 µl
4350-MC-100	UVssDNA mAb (clone C3B6)	100 µg
4354-MC-50	Anti-8-oxo-dG mAb (clone 2E2)	50 µl
4335-MC-100	Anti-PAR polymer mAb (clone 10HA)	100 µl
4336-BPC-100	Anti-PAR polymer polyclonal	100 µl
4338-MC-50	Anti-human/murine-PARP mAb (clone C2-10)	50 µg

FLARE[™] Assay Kits:

Catalog #	Description	Substrate	Size
4040-100-FK	Fpg Kit	8-oxoguanine	75 samples
4055-100-FK	T4-PDG Kit	Cis-syn isomers of cyclobutane pyrimidine dimers	75 samples
4065-100-FK	cv-PDG Kit	Cis-syn and trans-syn isomers of cyclobutane pyrimidine dimers	75 samples
4130-100-FK	hOGG1 Kit	8-oxoguanine, DNA containing formamidopyrimidine moieties	75 samples
4100-100-FK	UVDE Kit	Cyclobutane pyrimidine dimers, (6-4) photoproducts	75 samples
4045-01K-FK	Endonuclease III Kit	Thymine Glycol, 5,6-dihydrothymine 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, methyltartonyl urea, thymine ring saturated or fragmentation product	75 samples

Accessories:

Catalog #	Description	Size
4256-010-CC	CometAssay [®] Control Cells (alkaline assay)	1 set
4257-010-NC	Neutral CometAssay [®] Control Cells	1 set
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
3950-300-02	FLARE [™] Slides	100 slides
4040-100-FM	Fpg FLARE [™] Module	>100 samples
4130-100-FM	hOGG1 FLARE [™] Module	>100 samples
4045-100-FM	Endonuclease III FLARE [™] Module	>100 samples
4055-100-FM	T4-PDG FLARE [™] Module	>100 samples
4065-100-FM	cv-PDG FLARE [™] Module	>100 samples
3950-075-SP	FLARE [™] Sample Prep	>100 samples
4100-050-FM	UVDE FLARE [™] Module	>100 samples
4370-096-K	HT 8-oxo-dG ELISA Kit	96 wells

DNA Repair Enzymes:

Catalog #	Description	Size
4055-100-EB	T4 Endonuclease V (T4-Pyrimidine Dimer Glycosylase/ T4-PDG)	100,000 U
4150-010-EB	<i>Sulfolobus solfataricus</i> DNA Polymerase IV (Dpo4)	10 µg
4045-01K-EB	<i>E. coli</i> Endonuclease III	1000 U
4050-100-EB	<i>E. coli</i> Endonuclease IV	100 U
4130-100-EB	Human 8-oxo-G DNA Glycosylase (hOGG1)	100 U
4040-100-EB	<i>E. coli</i> Fpg	500 U
4025-100-EB	<i>E. coli</i> Uracil-N-Glycosylase	100 U
4000-500-EB	<i>E. coli</i> MutY DNA Glycosylase	500 U
4110-01K-EB	Human AP Endonuclease	1000 U
4020-01K-EB	Human β Polymerase	1000 U
4120-100-EB	Human FEN-1	100 U

Catalog #	Description	Size
4125-100-EB	<i>E. coli</i> Mismatch Uracil DNA Glycosylase (Mug)	100 U
4090-100-EB	Mouse 3-Methyladenine DNA Glycosylase (Aag)	100 U
4070-500-EB	Thermostable thymine mismatch DNA Glycosylase	500 U
4100-100-EB	<i>S. pombe</i> Ultraviolet DNA Endonuclease (UVDE)	100 µl
4065-100-EB	Chorella Virus Pyrimidine Dimer Glycosylase	1000 U

IX. Warning/Safety

The final Silver Staining solution (prepared in step V.iii) is considered hazardous material. Disposal should be performed according to local and state regulations. It is recommended to tap solution off the slide into a container for safe disposal. 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 of these chemical reagents. Trevigen assumes no liability for damage resulting from handling or contact with these products. MSDS sheets are available.

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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