

CrystalStain Cell Staining Solution

Troubleshooting Guide

50 mL, 100 mL

Cat No: CSS50, CSS100

Use this guide for common staining problems in the two workflows described for CrystalStain: single-cell colony assays and Transwell migration/invasion assays. The datasheet specifies methanol fixation, PBS washing, 20-minute CrystalStain incubation, and careful handling to avoid disrupting colonies or migrated cells.

Before You Start - Quick Checks

1. Store the staining solution at room temperature.
2. For colony assays, seed 2,000-5,000 cells per well in a 6-well plate and distribute cells individually.
3. For colony staining, fix with 1 mL methanol for 3 minutes, then wash with 1x PBS.
4. Stain with 1 mL CrystalStain for 20 minutes.
5. For Transwell assays, remove all cells remaining on the upper membrane surface before fixation/staining.
6. Use the datasheet-specified PBS wash steps before counting stained colonies or migrated cells.

Problem	Most likely cause	Recommended action
Colonies stain weakly	Fixation, staining time, or wash sequence may not match the colony protocol.	Fix colonies with 1 mL methanol for 3 minutes, wash with 1x PBS, stain with 1 mL CrystalStain for 20 minutes, then wash as specified.
Well background remains strongly blue	The post-stain washing step may be insufficient.	Wash with 1 mL 1x PBS for 5 minutes and repeat until the well surface appears pale blue, as stated in the colony protocol.
Colonies detach during processing	Medium, methanol, PBS, or stain may have been removed too aggressively.	Remove liquids carefully at each step. The datasheet repeatedly instructs users to avoid disrupting colonies.
Colonies are not individually separated	Initial seeding may be too dense or cells may not have been evenly distributed.	Seed 2,000-5,000 cells per well in 2 mL medium and ensure that cells are individually distributed across the 6-well surface.
Too few colonies are present at the planned endpoint	The culture may not yet contain individual colonies at the chosen treatment duration.	The datasheet gives example incubation periods of 7, 10, or 14 days and instructs users to ensure that individual colonies are present before final staining.
Colony assay results vary because medium conditions differ over time	The medium-refresh schedule may not have been kept consistent.	Refresh cell culture medium every 2-3 days as specified.

Table continues on next page.

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Troubleshooting Guide (continued)

Problem	Most likely cause	Recommended action
Transwell membrane shows cells on both surfaces	Cells remaining on the upper surface were not completely removed.	After incubation, clean the upper surface with a cotton swab and confirm that no cells remain there before fixing the migrated cells.
Very few migrated cells are visible	The Transwell seeding or chemoattractant conditions may differ from the datasheet protocol.	Use 20,000-40,000 cells in 250 uL serum-free medium in the top chamber and 500 uL complete medium containing 20% FBS in the lower chamber.
Migration signal varies between experiments	Incubation duration may not have been standardized.	Use a consistent 24- or 48-hour incubation as described in the Transwell protocol.
Migrated cells are lost during fixation	The membrane may have been handled too aggressively during methanol fixation and PBS washes.	Fix the lower-surface cells in methanol for 3 minutes and perform subsequent PBS handling carefully.
Transwell staining is weak	Staining time may be shorter than specified.	Incubate migrated cells with CrystalStain for 20 minutes after fixation and PBS washing.
Transwell background remains high	Upper-surface cells may remain or post-stain washing may be incomplete.	Remove upper-surface cells completely before staining and wash inserts with 1 mL 1x PBS for 5 minutes three times after staining.
Counts are difficult to compare across groups	Counting conditions may differ between wells or inserts.	Count colonies or migrated cells under an inverted microscope using the same counting approach for all comparison groups.

Still having trouble? Identify which workflow is being used first. For colony assays, recheck seeding density, 3-minute methanol fixation, 20-minute staining, and repeated PBS washing. For Transwell assays, confirm complete removal of upper-surface cells, the stated chemoattractant setup, fixation, staining, and three post-stain PBS washes.