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Directions for Use
Cytus™ Violet Cell Adhesion Stain

Cat# 5466-2EA

Product Overview

The Cytus™ Violet Cell Adhesion Stain is a ready-to-use, high-contrast staining reagent designed to support reliable visualization and quantification of adherent cells in cell adhesion and proliferation assays. Crystal Violet binds strongly to cellular DNA and proteins, producing an intense, uniform stain that highlights attached cells while nonadherent cells are removed during washing. This enables clear evaluation of cell attachment, spreading, and overall adhesion performance across a variety of substrates, including biomaterials, extracellular matrix coatings, and stiffness-tunable platforms such as CytoSoft™.

This staining solution offers a simple, reproducible workflow suitable for mechano-biology research, biomaterial screening, and high-throughput adhesion studies. When paired with automated imaging systems, the Cytus™ Violet Cell Adhesion Stain provides enhanced contrast and consistent staining performance, enabling efficient, high-content analysis of cell adhesion behaviors across diverse experimental conditions.

Characterization and Testing

Parameter/Tests	Specification
Concentration	0.1% w/v in water
Appearance	Dark purple
Sterility	No growth
Cell staining	Characteristic
Volume	2 x 25 ML
Storage	25 °C

Materials Needed

- Cytus™ Violet Cell Adhesion Stain (Cat# 5466-2EA)
- Cells/cell lines of interest
- Cell culture media
- Sterile 1X PBS
- Cell culture plates or CytoSoft™ Rigidity Plates
- Coating solutions, i.e. PureCol® (Cat #5005)
- Centrifuge tubes
- Serological pipettes
- Micropipette and tips
- Orbital shaker
- Fluorescence microscope
- Plate reader

Sample Staining Procedure

1. Follow the CytoSoft™ Coating Procedure to coat each CytoSoft™ plate with diluted extracellular matrix protein prior to cell addition (i.e. diluted PureCol solution). For non-coated standard tissue culture plates, proceed to Step #2 for direct cell seeding.

2. Seed and culture cells according to manufacturer's protocol for desired time.

3. Remove cell culture medium and wash/rinse the cell monolayer with sterile 1X PBS twice. Aspirate the PBS.

4. **Staining**: add sufficient Cytus™ Violet to cover the wells and incubate the plates away from direct light at room temperature for 20 to 30 minutes.

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Well Plate Type	Volume of Cytus™ Violet Cell Adhesion Stain per Well
6-Well	1 to 3 mL
12-Well	1 to 2 mL
24-Well	0.5 to 1 mL
48-Well	200 to 400 µL
96-Well	100 to 200 µL

5. Remove the Cytus™ Violet and rinse the stained wells thoroughly with deionized water until no residual dye remains.

6. Air dry the stained plates completely without the lids at room temperature overnight.

7. **Imaging:** Image the stained plates using the brightfield or fluorescence (Ex/Em: 592/636 nm, i.e. Cy5) filter.

8. **Plate reading:** Load the stained plate into a microplate reader and measure the signal using either fluorescence mode (Ex/Em: 592/636 nm) or absorbance mode (590 nm).

Sample images

Fig.1. Human fibroblasts cultured in a CytoSoft™ 24-Well Imaging Plate.

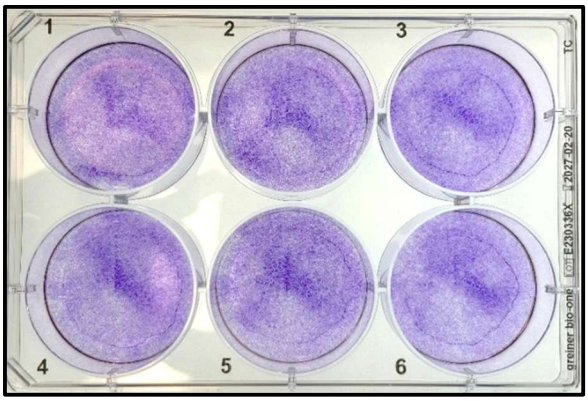

Fig.2. Human fibroblasts cultured in a 6-well CytoSoft™ 6-Well Plate.

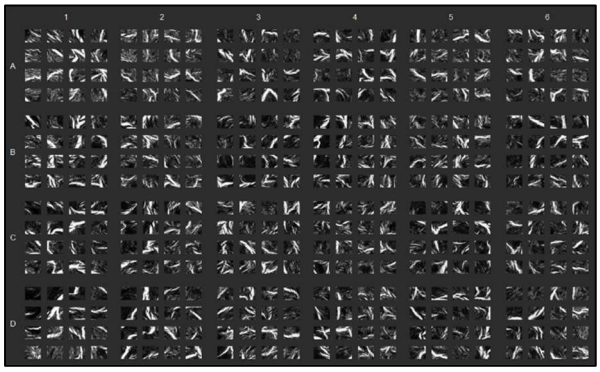

Fig.3. High throughput and automated cell image acquisition.

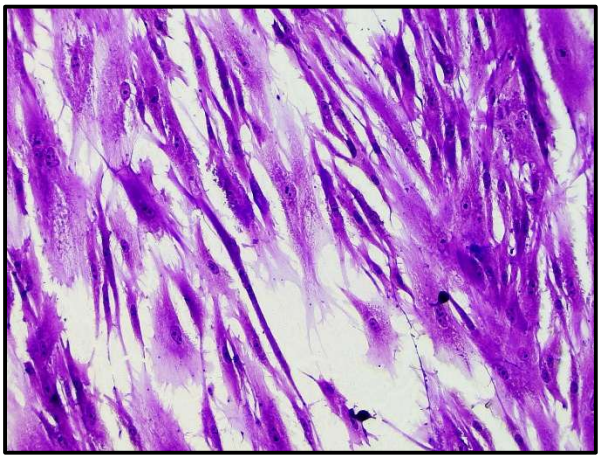

Fig.4. Magnified 20× image showing cell adhesion of human fibroblasts cultured in CytoSoft™ Imaging plates.

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	1	2	3	4	5	6	7	8	9	10	11	12
A	0.314969	0.318162	0.348485	0.354300	0.351723	0.381423	0.333431	0.331954	0.344885	0.361731	0.373431	0.361438
B	0.306385	0.329838	0.362777	0.368162	0.329200	0.355931	0.356508	0.354715	0.369323	0.396700	0.357131	0.345492
C	0.300800	0.327054	0.341900	0.343554	0.350638	0.371515	0.353800	0.338092	0.357785	0.376392	0.374092	0.347315
D	0.293631	0.318546	0.351085	0.346554	0.339077	0.324208	0.326269	0.298915	0.337977	0.376262	0.370315	0.372031
E	0.317131	0.315215	0.369492	0.381431	0.369900	0.351338	0.343538	0.313992	0.326877	0.348815	0.344638	0.334377
F	0.303500	0.303362	0.383169	0.379138	0.381438	0.364762	0.353531	0.334538	0.327977	0.324200	0.308615	0.322377
G	0.320400	0.323054	0.355562	0.368085	0.372862	0.364492	0.323792	0.314192	0.325946	0.312331	0.303731	0.266238
H	0.270531	0.307400	0.307123	0.314400	0.328792	0.305238	0.280500	0.249246	0.289385	0.292662	0.259385	0.204369

Fig.5. Representative heat map of a 96-well cell culture plate showing Cytus™ Violet-stained cells. Each cell in the heat map corresponds to an individual well and represents the average absorbance reading of the Cytus™ Violet Cell Adhesion Stain (n = 9 readings per well). Color intensity reflects absorbance values, illustrating well-to-well signal distribution and assay uniformity across the plate.

