CrystalStain, Cell Staining Solution

50 ml, 100 ml

Cat No: CSS50, CSS100

Shipping : Ship at ambient temperature.
Storage : Store at room temperature.

General Information

CrystalStain, Cell Staining Solution is a frequently used reagent to visualize cells in cell culture applications like single cell colony assay, Transwell migration assay or Transwell invasion assay.

Recommended protocol for single cell colony assay

1. Seed 2000-5000 cells per well in 2 ml cell culture medium in a 6-well plate. Incubate cells at 37°C in a humidified CO2 incubator for 24 hours. Ensure that cells are individually distributed all over the well.

2. Apply various concentrations of substances to be tested to the cells.

3. Incubate the cells for an appropriate length of time (e.g. 7, 10, 14 days). Ensure the presence of individual colonies within the wells.

4. Refresh the cell culture medium every 2-3 days.

5. To finalize the experiment, remove the medium carefully and wash the wells with 1x PBS twice.

6. Add 1 ml methanol to each well and incubate for 3 minutes. Remove methanol carefully without disrupting colonies.

7. Add 1 ml 1x PBS to each well and incubate for 1 minute. Remove 1x PBS carefully without disrupting colonies.

8. Add 1 ml CrystalStain, Cell Staining Solution to each well and incubate for 20 minutes. Remove CrystalStain, Cell Staining Solution carefully without disrupting colonies.

9. Add 1 ml 1x PBS to each well and incubate for 5 minutes. Repeat this step until the well surface looks pale blue.

10. Count the total number of colonies and the number of paraclones, meroclones and holoclones* in each well under an invert microscope.

*Holoclones are densely packed bundles of cells, which are spherical in shape, whereas paraclones are made up of larger dispersed cells. Meroclones has an intermediate feature with a dichotomy of cell shapes and sizes (1).

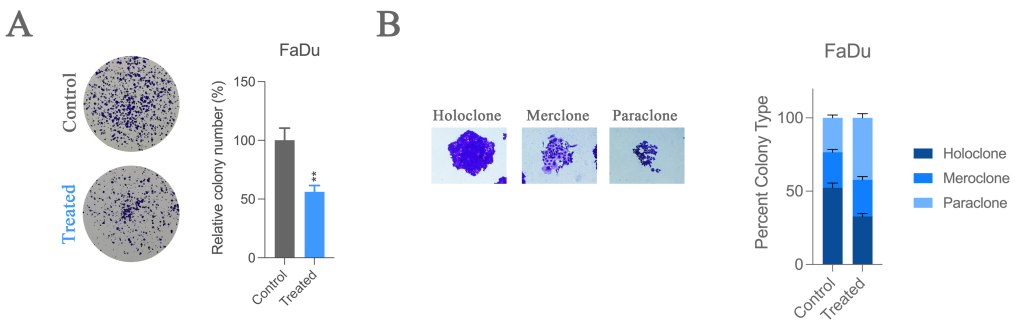

Figure 1. A. The number of single colonies formed by FaDu cell treated with a chemotherapeutic agent. B. The distribution of colony types formed by FaDu cell treated with a chemotherapeutic agent.

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Recommended protocol for Transwell migration/invasion assay

1. Seed 20000-40000 cells resuspended in 250 µL serum-free medium into the top chamber at least in duplicates per group.

2. Add 500 µL complete medium containing 20% FBS into the lower chamber as chemoattractant.

3. Incubate cells at 37°C in a humidified CO2 incubator for 24 or 48 hours.

4. After incubation, clean cells that remained on the upper surface of the membranes using a cotton swab. Ensure that no cells remained on the upper surface of the membranes.

5. Add 1 ml methanol to wells of a 24 well plate and fix the cells that migrated to the lower surface of the membranes with methanol for 3 minutes.

6. Add 1 ml 1x PBS to wells of a 24 well plate and wash the cells that migrated to the lower surface of the membranes with 1x PBS for 1 minute.

7. Add 1 ml CrystalStain, Cell Staining Solution to wells of a 24 well plate and incubate the cells that migrated to the lower surface of the membranes for 20 minutes.

9. Wash inserts within the wells with 1 ml 1x PBS for 5 minutes 3 times.

10. Count the total number of cells that migrated to the lower surface of the membranes under an invert microscope.

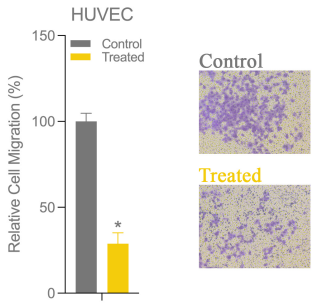

Figure 2. Relative migration of HUVEC cells treated with a chemotherapeutic agent.

References

1. L. Flynn, M.P. Barr, A.M. Baird, P. Smyth, O.M. Casey, G. Blackshields, J. Greene, S.R. Pennington, E. Hams, P.G. Fallon, J. O'Leary, O. Sheils and S.P. Finn, Prostate cancer-derived holoclones: a novel and effective model for evaluating cancer stemness. Sci. Rep. **10**, 11329 (2020)

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