Supplementary Material: Efficient and consistent dissociation of adherent cell cultures using TrypsiNNex®

General methods

Two-dimensional (2D) cultures

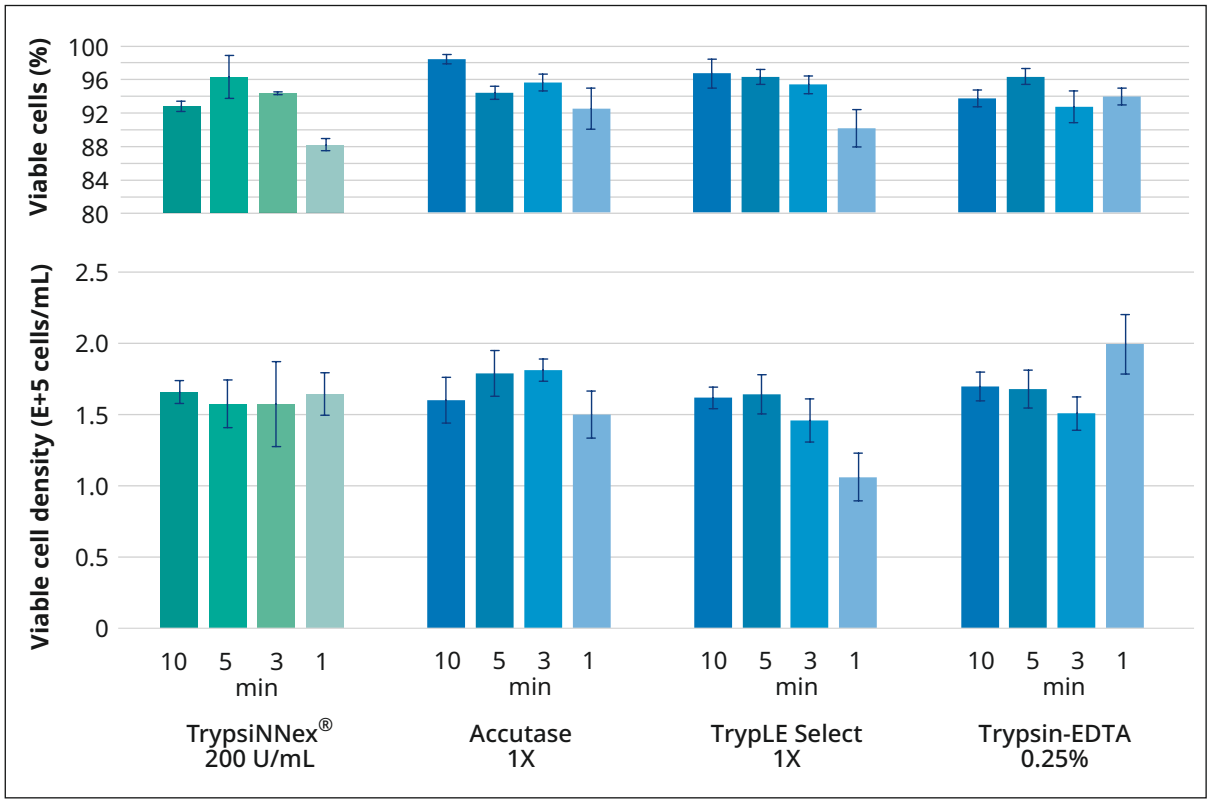
Approximately 1–1.2E+5 cells were seeded into 12-well plates with 1 mL culture medium and allowed to reach 90% confluence. Before dissociation, all wells were rinsed with phosphate buffer saline (PBS). Depending on the cell line, different incubation times and trypsin concentrations were tested in triplicate. The plates were incubated at 37°C, and trypsinization was stopped with 1 mL culture medium. The resulting cell suspension was collected, centrifuged, and resuspended in 200 µL medium for analysis. Cell counting and viability data were recorded with a NucleoCounter® NC-200™ (Chemometec, Denmark).

Three-dimensional (3D) cultures

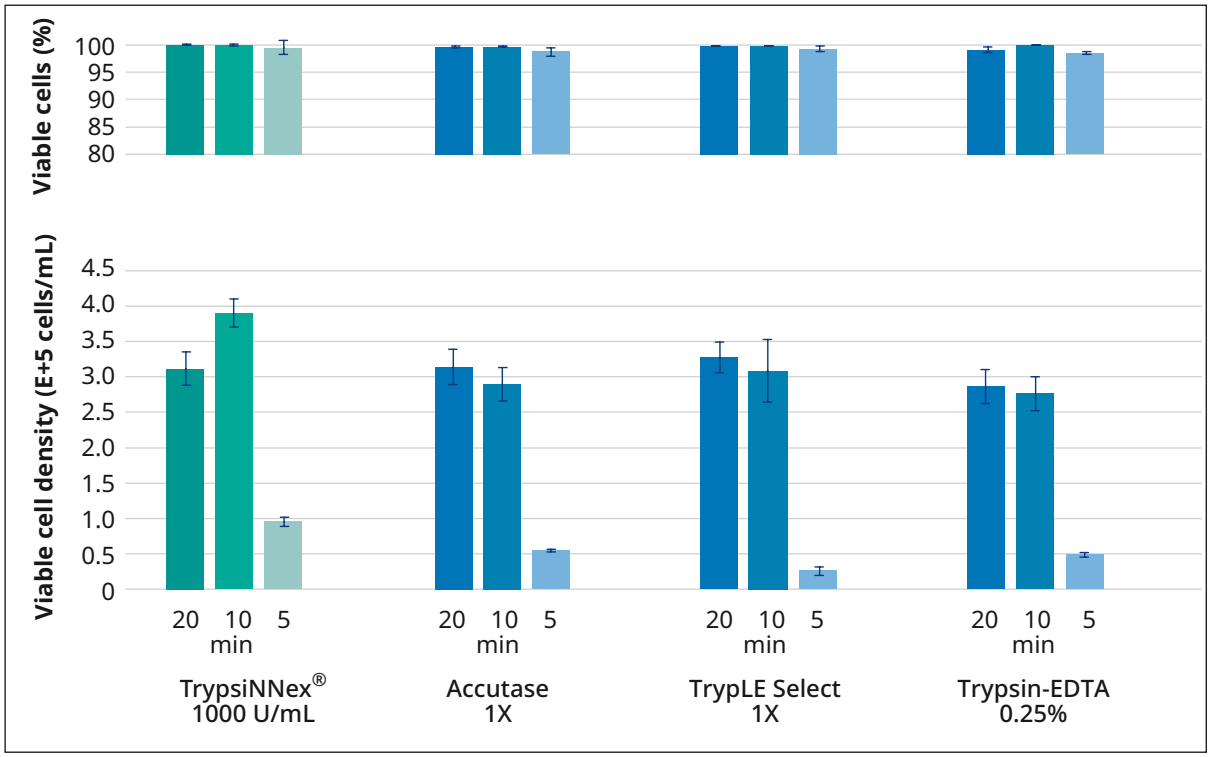
Cells were seeded on PET-based macrocarriers placed in wells of a 12-well plate. The cells were allowed to reach 90% confluence before rinsing them with PBS and adding the trypsin product to be tested. Depending on the cell line, different concentrations were tested in triplicate. All 3D culture plates were incubated at 37°C for 30 minutes before stopping trypsinization with culture medium. The resulting cell suspension was collected, centrifuged, and resuspended in 1 mL medium for analyses. Cell counting and viability data were recorded with a NucleoCounter® NC-200™ (Chemometec, Denmark).

Experiment results by cell line and culture type (2D, 3D)

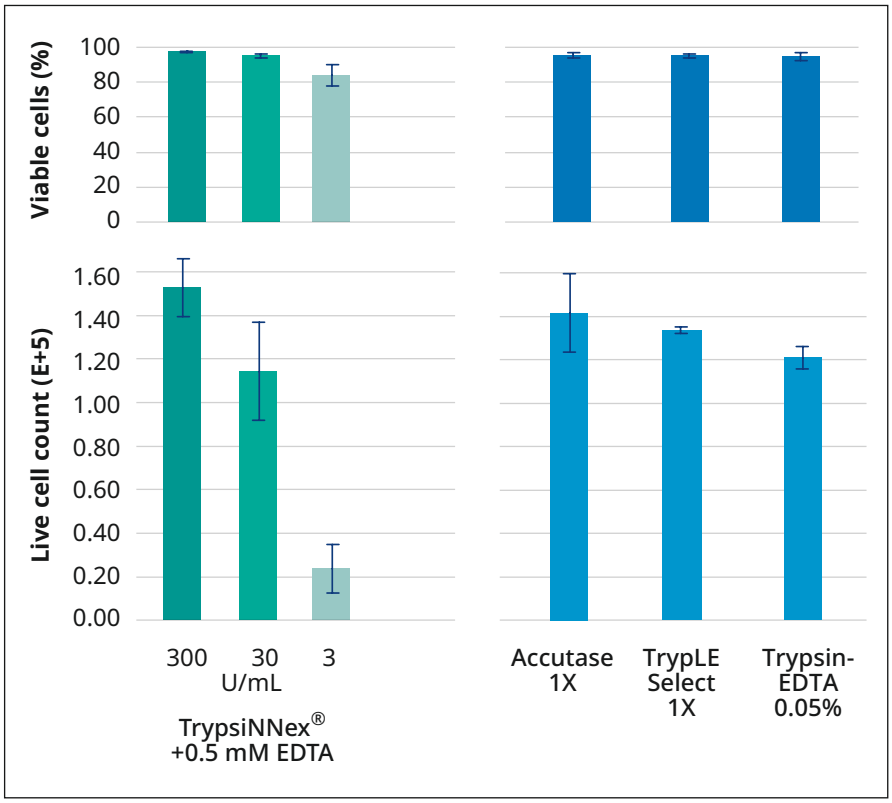
Human embryonic kidney cells (HEK293) – 2D



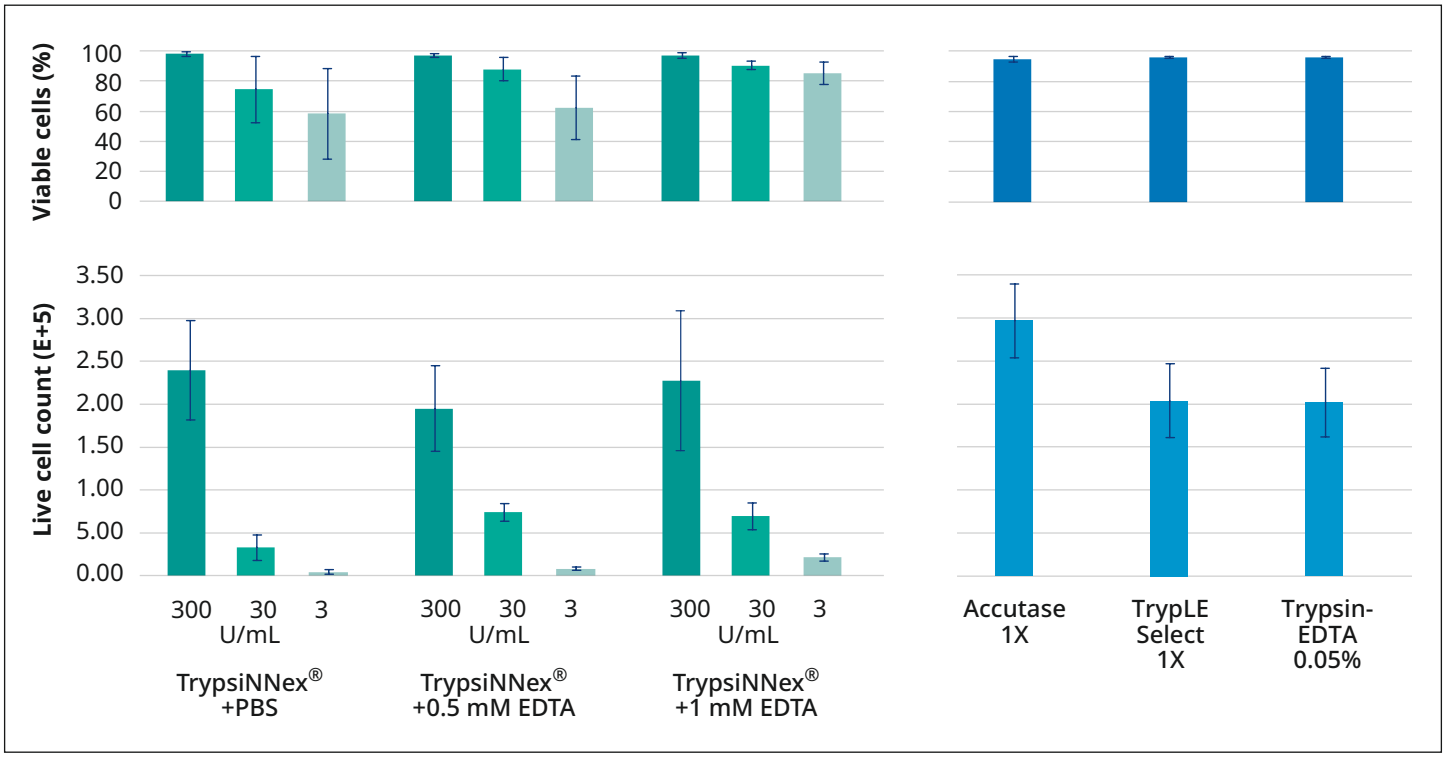
Madin-Darby canine kidney cells (MDCK) – 2D



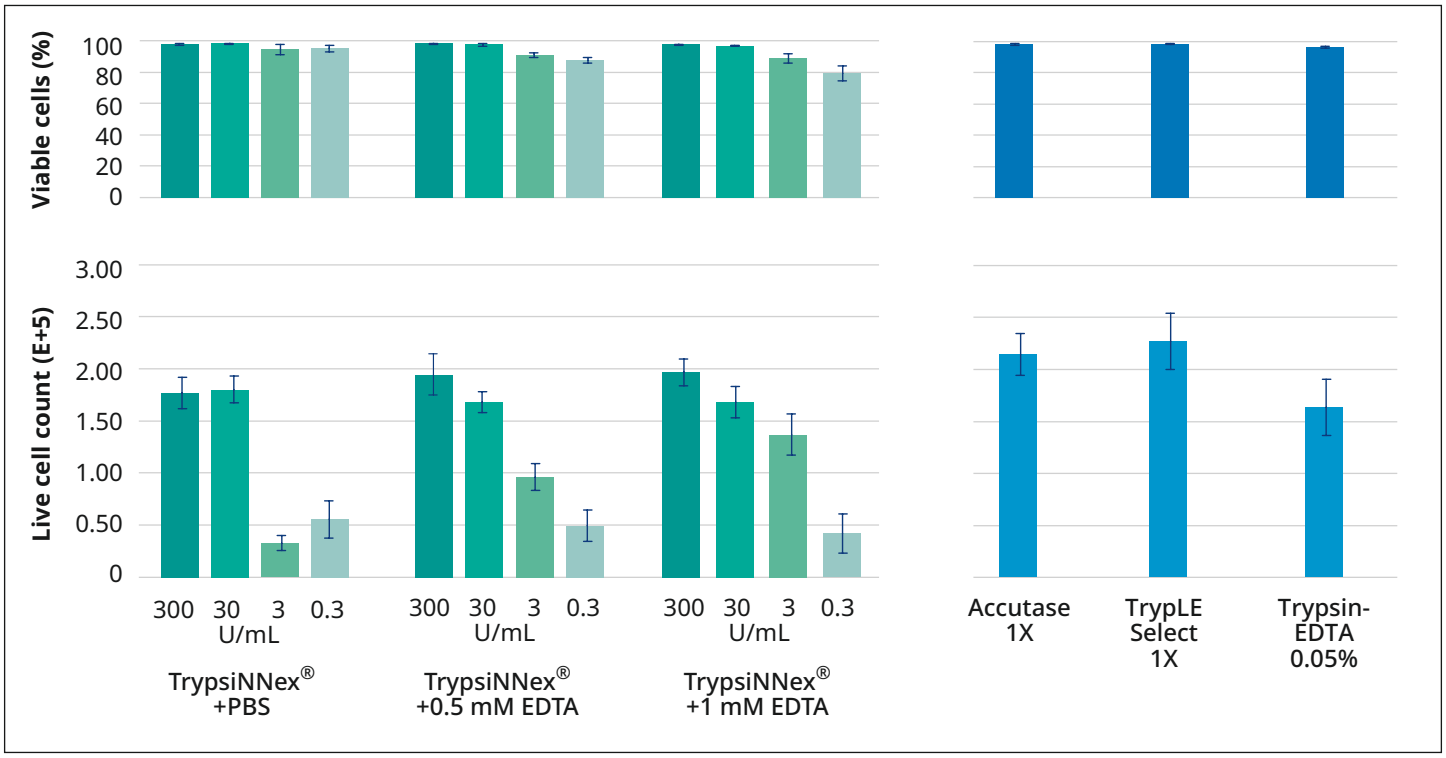
Medicinal Research Council strain 5 cells (MRC5) – 2D, 4-minute incubation



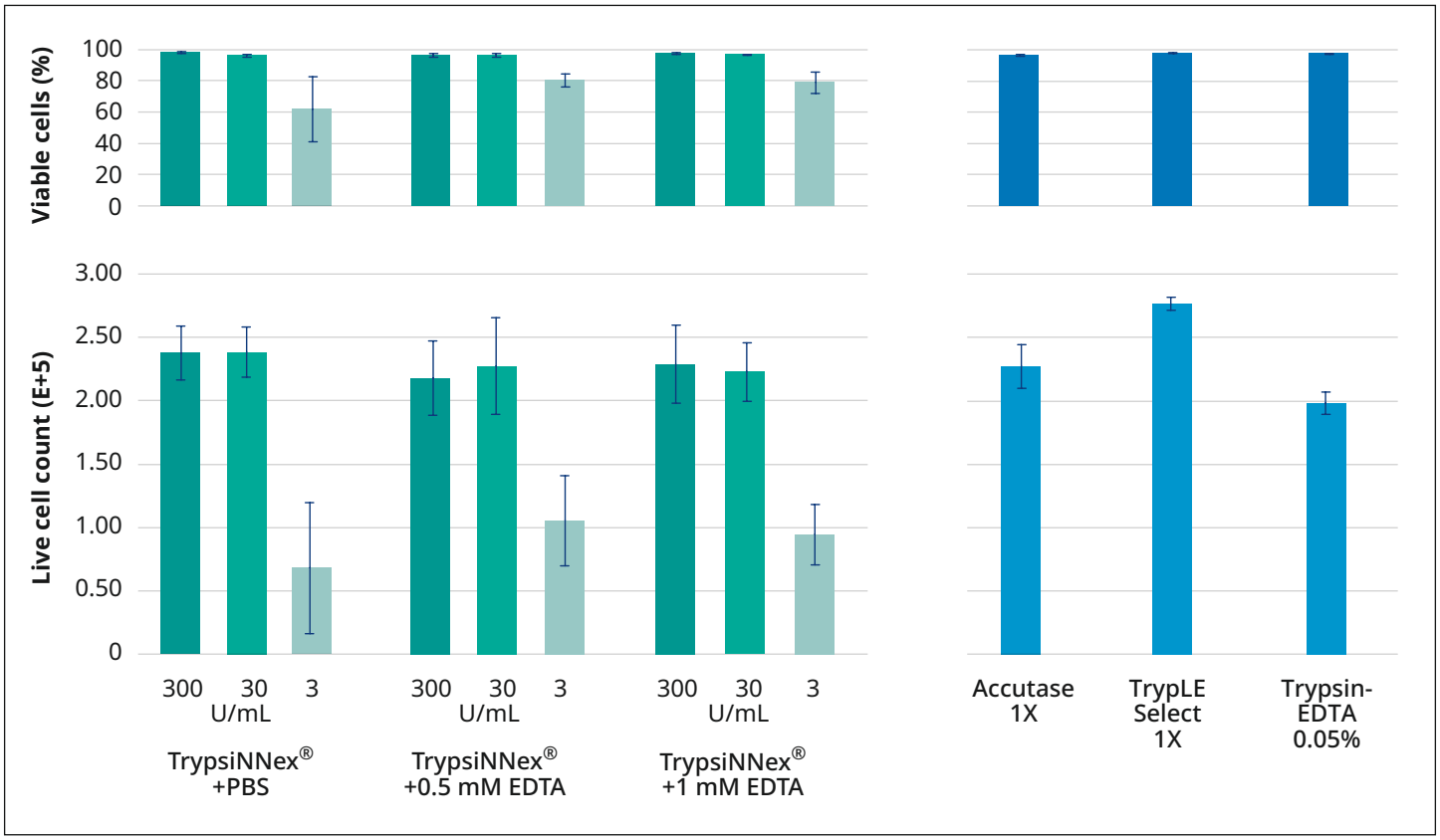
MRC5 cells – 3D, 30-minute incubation



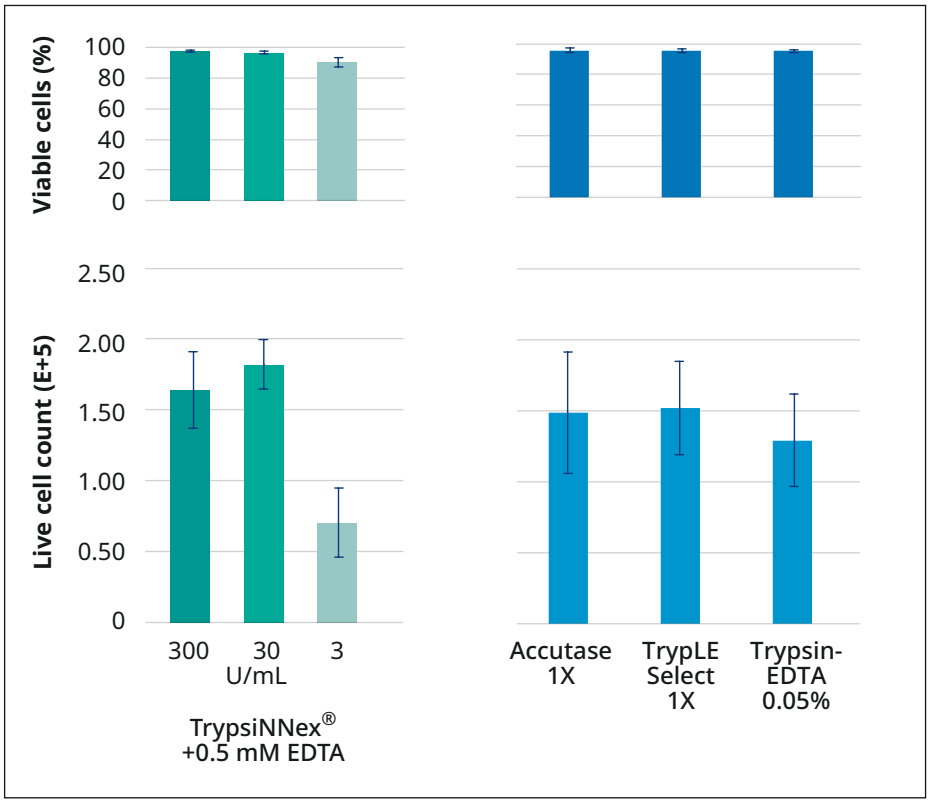
Bone marrow-derived mesenchymal cells (BM-MSC) – 2D, 2-minute incubation



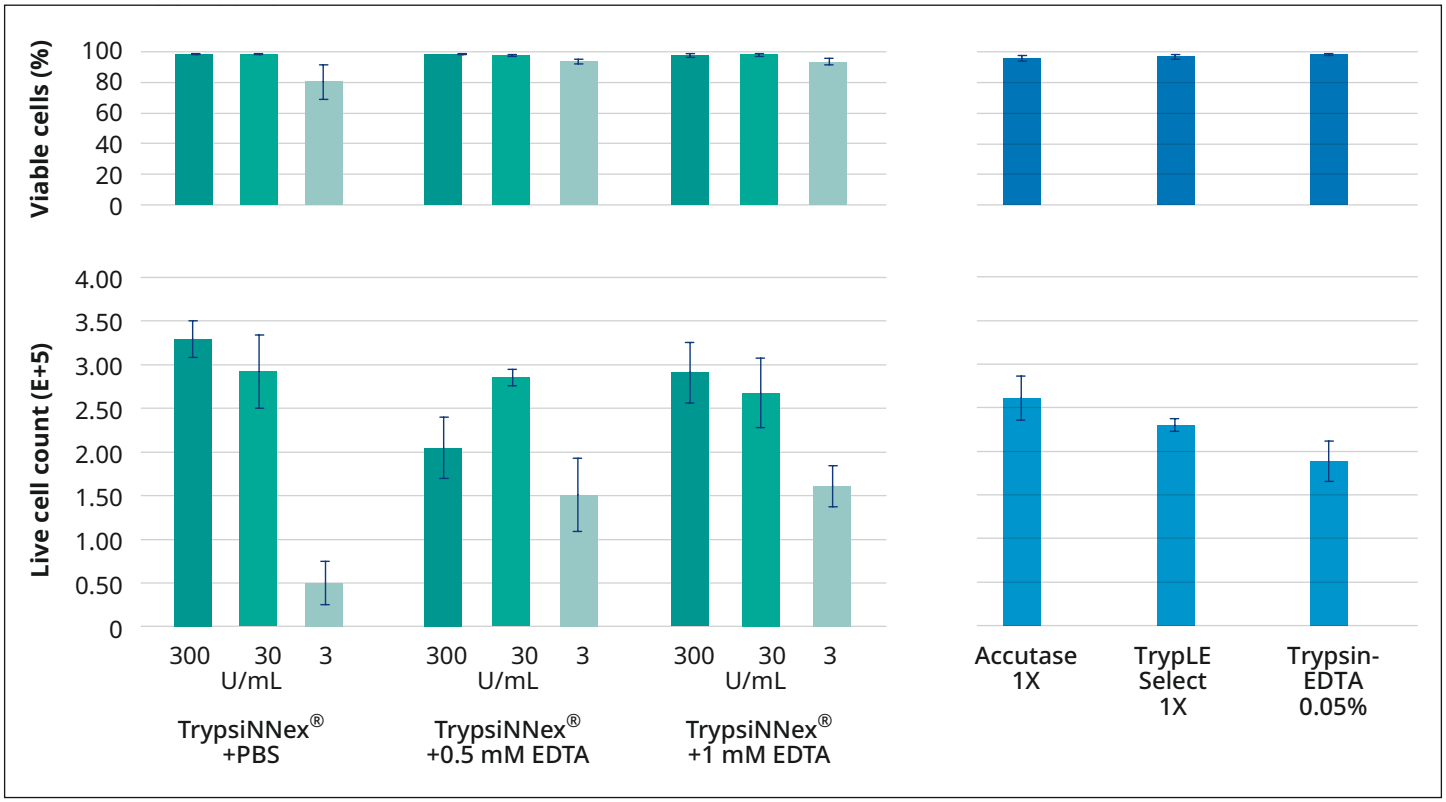
BM-MSC – 3D, 30-minute incubation



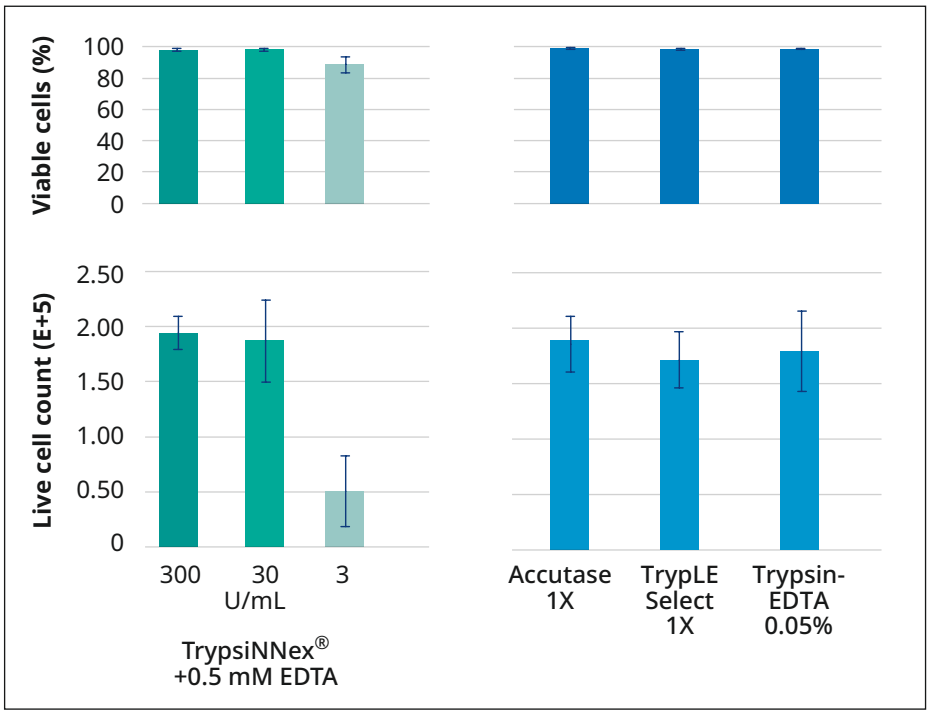
Umbilical cord-derived mesenchymal cells (UC-MSC) – 2D, 2-minute incubation



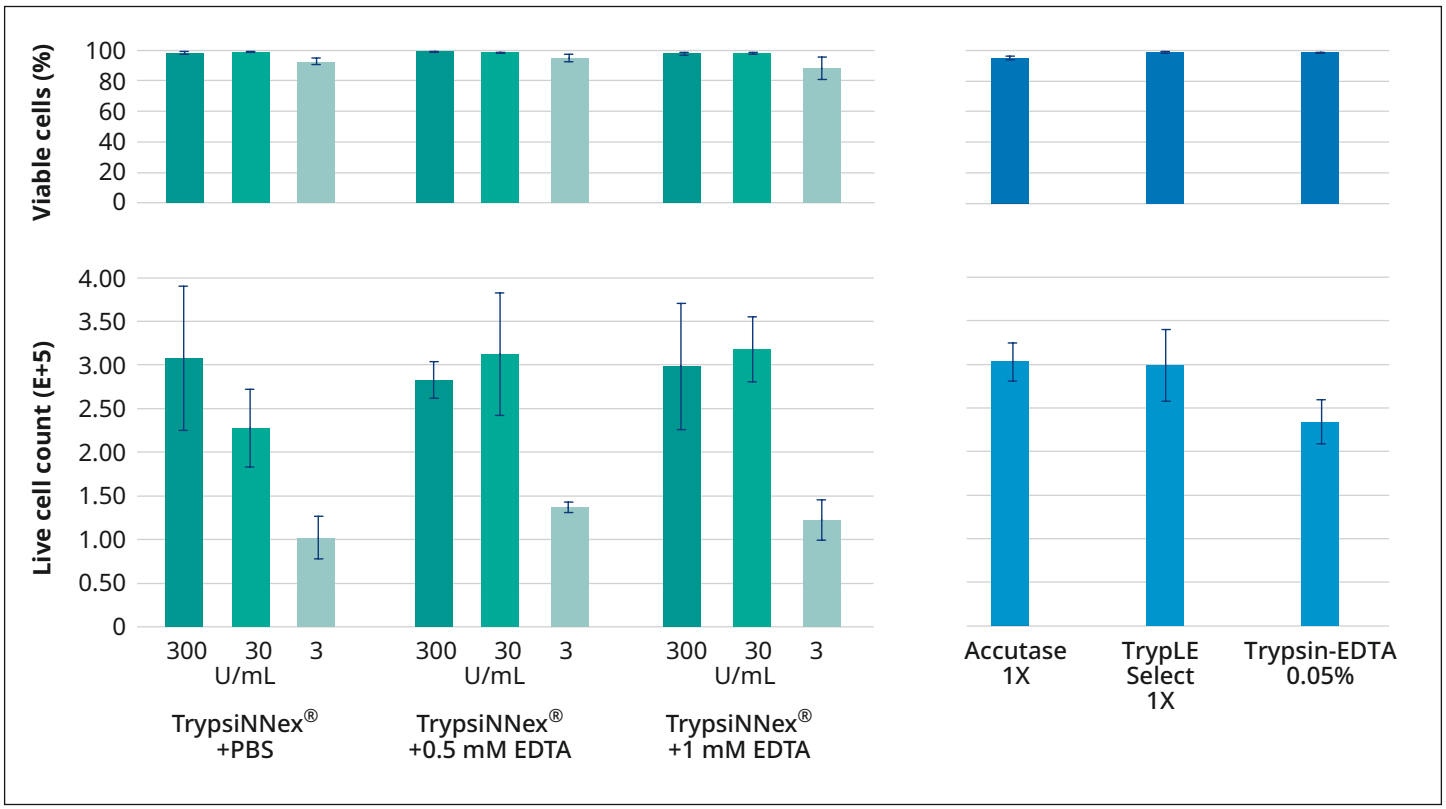
UC-MSC – 3D, 30-minute incubation



Adipose-derived mesenchymal cells (AD-MSC) – 2D, 2-minute incubation



AD-MSC – 3D, 30-minute incubation



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