CloneR™

Supplement for the Increased Cloning Efficiency and Single-Cell Survival of hPSCs

CloneR™ is a defined, serum-free supplement formulated for greatly enhancing the cloning efficiency and single-cell survival of human pluripotent stem cells (hPSCs), especially under clonal and low-density seeding conditions. Designed for use in feeder-free culture systems, this flexible supplement is compatible with either mTeSR™1 or TeSR™-E8™ maintenance medium, a range of cell culture matrices, and human embryonic stem (hES) and induced pluripotent stem (hiPS) cell lines. Unlike current methods, CloneR™ enables the robust generation of clonal hPSC lines without single-cell adaptation, thus minimizing the risk of acquiring genetic abnormalities.

PRODUCT	SIZE	CATALOG #
CloneR™	10 mL	05888
	5 x 10 mL	05889

Advantages:

EFFICIENT. Increased single-cell survival at low and clonal densities.

EASY-TO-USE. No adaptation to single-cell passaging required.

FLEXIBLE. Compatible with any TeSR™ maintenance medium and your choice of cell culture matrix.

ROBUST. Increased cloning efficiency across multiple hES and hiPS cell lines.

DEFINED. Serum-free and feeder-free formulation.

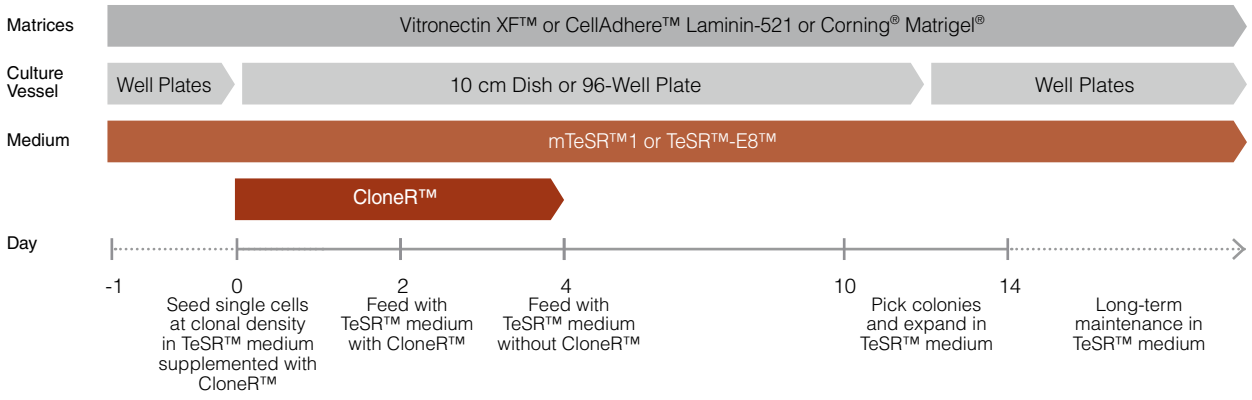

Figure 1. hPSC Single-Cell Cloning Workflow with CloneR™

On day 0, hPSCs are seeded as single cells at clonal density (e.g. 25 cells/cm^2) or sorted at 1 cell per well in 96-well plates in mTeSR™1 (Catalog #85850) or TeSR™-E8™ (Catalog #05940) medium supplemented with CloneR™. On day 2, the cells are fed with TeSR™ medium containing CloneR™ supplement. From day 4, cells are maintained in TeSR™ medium without CloneR™. Colonies are ready to be picked between days 10 - 14. Clonal cell lines can be maintained long-term in TeSR™ medium. Culture vessels shown are suggestions.

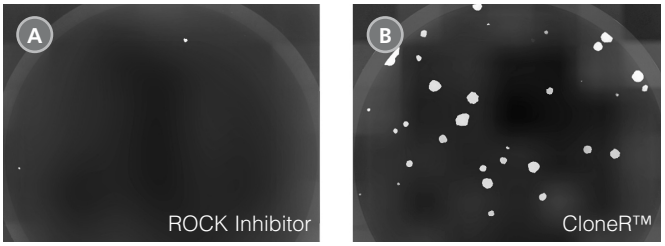

Figure 2. CloneR™ Increases the Cloning Efficiency of hPSCs at Low Seeding Densities

hPSCs plated in mTeSR™1 supplemented with CloneR™ demonstrated significantly increased cloning efficiencies compared to cells plated in mTeSR™1 containing ROCK inhibitor (10µM Y-27632). Shown are representative images of alkaline phosphatase-stained colonies at day 7 in individual wells of a 12-well plate. H1 hES cells were seeded at clonal density (100 cells/well, 25 cells/cm^2) in mTeSR™1 supplemented with (A) ROCK inhibitor or (B) CloneR™ on Vitronectin XF™ cell culture matrix.

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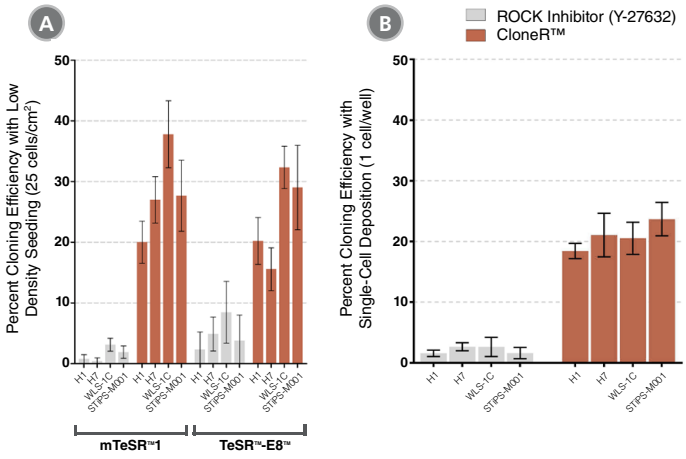

Figure 3. CloneR™ Increases the Cloning Efficiency of hPSCs and is Compatible with Multiple hPSC Lines and Seeding Protocols

TeSR™ medium supplemented with CloneR™ increases hPSC cloning efficiency compared with cells plated in TeSR™ containing ROCK inhibitor (10μM Y-27632). hES (H1 and H7) and hiPS (WLS-1C and STiPS-M001) cells were seeded (A) at clonal density (25 cells/cm²) in mTeSR™1 and TeSR™-E8™ and (B) by single-cell deposition using FACS (seeded at 1 cell/well) in mTeSR™1.

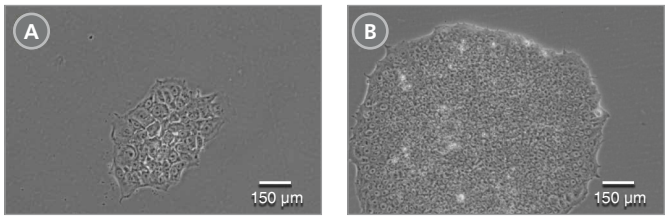

Figure 4. CloneR™ Yields Larger Single-Cell Derived Colonies

hPSCs seeded in mTeSR™1 supplemented with CloneR™ result in larger colonies than cells seeded in mTeSR™1 containing ROCK inhibitor. Shown are representative images of hPSC clones established after 7 days of culture in mTeSR™1 supplemented with (A) ROCK inhibitor or (B) CloneR™.

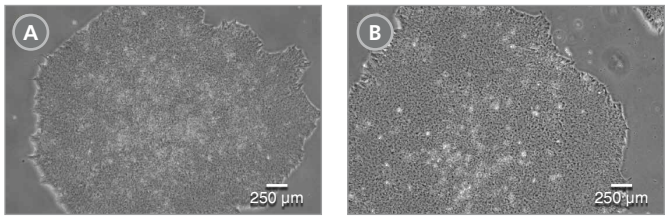

Figure 5. Clonal Cell Lines Established Using CloneR™ Display Characteristic hPSC Morphology

Clonal cell lines established using mTeSR™1 or TeSR™-E8™ medium supplemented with CloneR™ retain the prominent nucleoli and high nuclear-to-cytoplasmic ratio characteristic of hPSCs. Representative images at passage 7 after cloning are shown for clones derived from the parental (A) H1 hES cell and (B) WLS-1C hiPS cell lines.

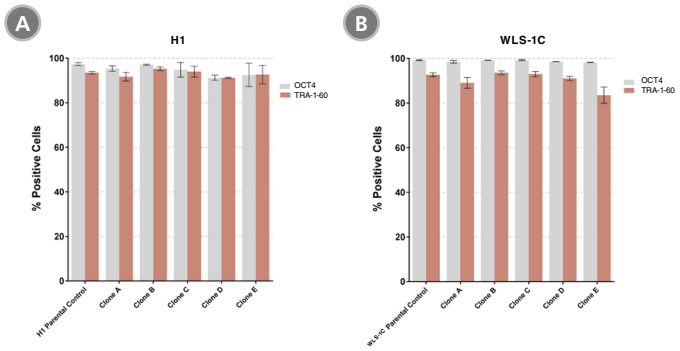

Figure 6. Clonal Cell Lines Established with CloneR™ Express High Levels of Undifferentiated Cell Markers

hPSC clonal lines established using mTeSR™1 supplemented with CloneR™ express comparable levels of undifferentiated cell markers, OCT4 (Catalog #60093) and TRA-1-60 (Catalog #60064), as the parental cell lines. (A) Clonal cell lines established from parental H1 hES cell line. (B) Clonal cell lines established from parental WLS-1C hiPS cell line. Data is presented between passages 5 - 7 after cloning and is shown as mean ± SEM; n = 2.

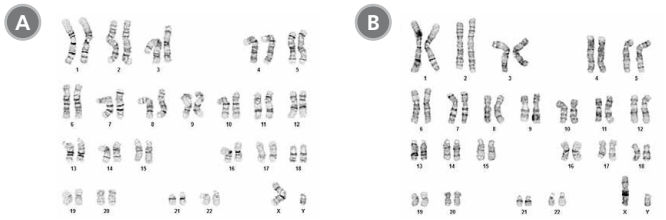

Figure 7. Clonal Cell Lines Established Using CloneR™ Display a Normal Karyotype

Representative karyograms of clones derived from parental (A) H1 hES cell and (B) WLS-1C hiPS cell lines demonstrate that the clonal lines established with CloneR™ have a normal karyotype. Cells were karyotyped 5 passages after cloning, with an overall passage number of 45 and 39, respectively.

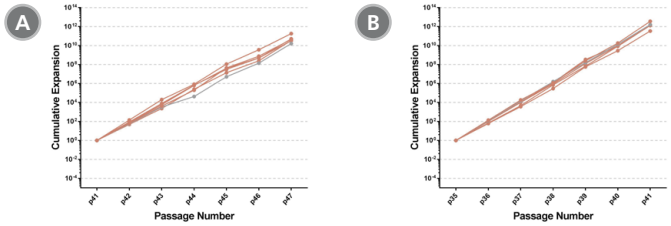

Figure 8. Clonal Cell Lines Established Using CloneR™ Display Normal Growth Rates

Fold expansion of clonal cell lines display similar growth rates to parental cell lines. Shown are clones (orange) and parental cell lines (gray) for (A) H1 hES cell and (B) WLS-1C hiPS cell lines.