

Easy and Reliable Passaging of hPSC Cultures Without Manual Selection and Scraping

Standard human pluripotent stem cell (hPSC) passaging protocols are laborious, involving manual removal of differentiated cells by aspiration or scraping, dissociation to loosen adherent colonies from the surface, and finally scraping the cultured cells off of the surface as cell aggregates. Simpler, single-cell hPSC passaging protocols have been evaluated, though aggregate-based passaging is generally recommended because repeated passaging as single cells can lead to the accumulation of chromosomal abnormalities.¹

ReLeSR™ is an enzyme-free reagent for dissociation and passaging of hPSCs as aggregates without manual selection or scraping. Passaging hPSCs with ReLeSR™ easily generates optimally-sized aggregates, while eliminating the hassle and variability associated with manual manipulation. By eliminating the need for scraping, ReLeSR™ enables the use of culture flasks and other closed vessels, thus facilitating culture scale-up and automation.



Advantages of ReLeSR™:

- Chemically-defined, enzyme-free formulation
- Selectively detaches undifferentiated cells
- Easily generates optimally-sized aggregates without manual scraping
- Compatible with culture processes involving closed vessels
- Compatible with mTeSR™1 and TeSR™-E8™

Passaging Protocol Comparison

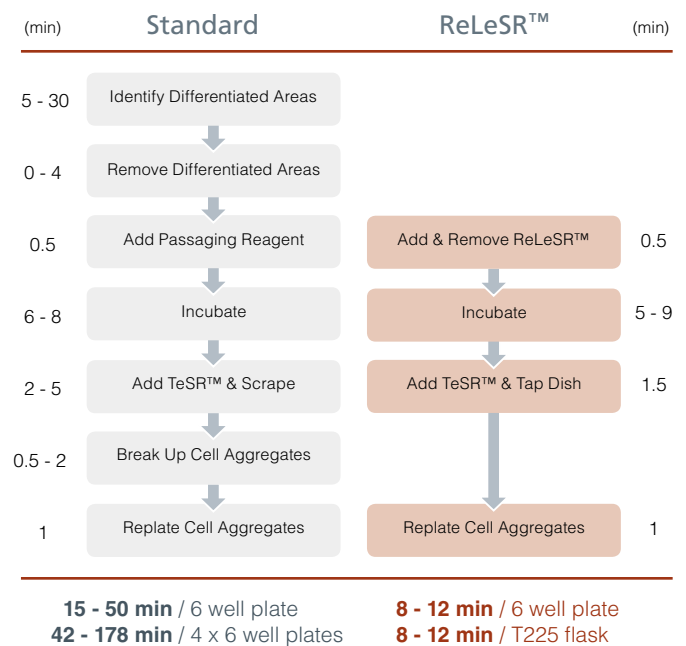


FIGURE 1. ReLeSR™ passaging protocol eliminates difficult and time-consuming steps, thereby enabling easy culture scale-up.

Surface area of 4 x 6 well plates (230 cm²) is comparable to that of a T225 flask (225 cm²). TeSR™ = TeSR™ family media (mTeSR™1, TeSR™-E8™).

Selectively Detach Undifferentiated Cells

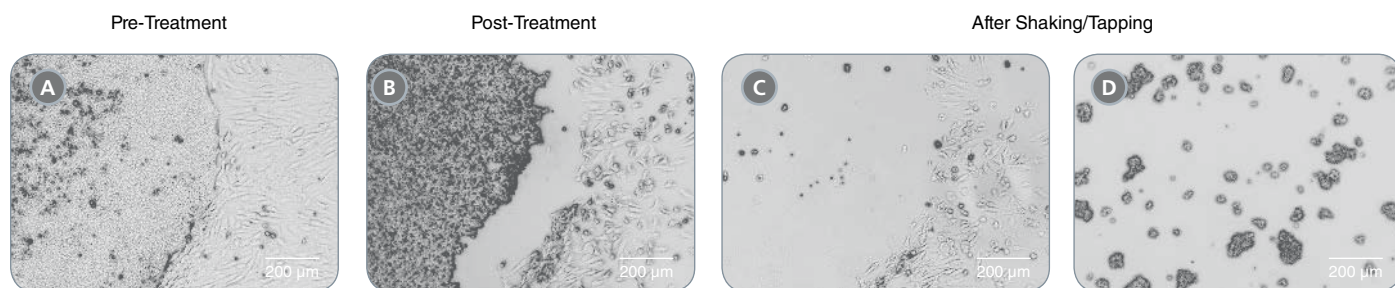


FIGURE 2. ReLeSR™ selectively detaches undifferentiated cells from pluripotent stem cell cultures without manual selection. Optimally-sized aggregates are generated following shaking/tapping of the cultureware.

(A) An hPSC culture ready for passaging. Note the presence of differentiated cells at the edge of the undifferentiated hPSC colony. (B) Following incubation with ReLeSR™, the undifferentiated hPSC colony starts to lift off of the cultureware. The differentiated cells remain attached to the cultureware. (C) Following shaking/tapping of the cultureware, the undifferentiated cells completely lift off of the cultureware. (D) The undifferentiated hPSC colony is broken up into optimally-sized aggregates for replating.

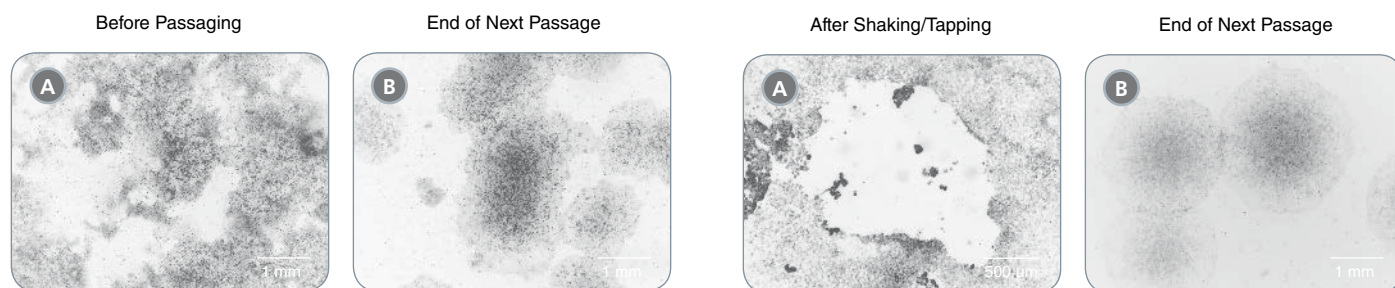


FIGURE 3. Poor quality human pluripotent stem cell cultures containing large proportions of differentiated cells can be rescued by passaging with ReLeSR™.

(A) A poor quality hPSC culture containing ~50% undifferentiated cells. (B) Following passaging with ReLeSR™, the differentiated cells have largely been eliminated from the culture, with >90% undifferentiated cells present at the end of the next passage.

FIGURE 4. Easily isolate newly generated human iPS cell colonies with ReLeSR™ by selectively detaching undifferentiated cells and leaving non-reprogrammed cells behind.

(A) A TeSR™-E7™ reprogramming culture which has been treated with ReLeSR™ to detach the putative iPS cell colony, leaving the non-reprogrammed and differentiated cells behind. (B) Cultures contain a high proportion of undifferentiated cells by the end of the first passage.

Product Information

PRODUCT	SIZE	CATALOG #
ReLeSR™	100 mL	05872
	500 mL	05873

References

1. Mitalipova et al. Preserving the genetic integrity of human embryonic stem cells. *Nat. Biotechnol.* 23(1) 19-20, 2005.

For a complete list of related products, including specialized cell culture and storage media, matrices, antibodies, cytokines and small molecules, visit www.stemcell.com/hPSCworkflow or contact us at techsupport@stemcell.com.