StemAdhere™ User Protocol

StemAdhere™ Pluripotency Substrate (Product No. S2071-500UG)

- 500 μg StemAdhere Pluripotency Substrate, sterile filtered, 250 μg/ml
  Stability and storage: 6 months at -80 °C or 2 weeks at 4 °C, undiluted

StemAdhere™ Pluripotent Cell Growth Kit Components (Product No. S2112)

- 500 μg StemAdhere Pluripotency Substrate, sterile filtered, 250 μg/ml
  Stability and storage: 6 months at -80 °C or 2 weeks at 4 °C, undiluted
- 100 ml Dilution Buffer, sterile filtered
  Stability and storage: 6 months at 15-30 °C. Do not refrigerate.
- 100 ml Cell Release Buffer, sterile filtered
  Stability and storage: 6 months at 15-30 °C
- 8 ea 6-well polystyrene plates, non-TC treated, sterile individually wrapped

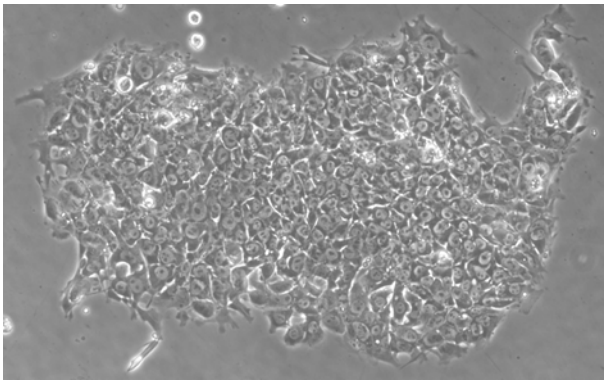

Fig. 1. Colony of H1 cells growing on StemAdhere™ coated non-TC treated 24-well plate

StemAdhere™ Pluripotency Substrate is a defined single-component cell attachment factor intended to support proliferation of human stem cells (ES and iPS) and maintenance of the pluripotent state [1, 2]. StemAdhere consists entirely of human sequences, and is to be used for coating of sterile, non-tissue culture treated polystyrene dishes and plates. Coating should be performed on the day of use; plates with coating solution may also be stored overnight at 4 °C. Cells grown on StemAdhere exhibit a flatter and larger cell morphology than seen on commonly used matrices. This morphology is indicative of greater cell adhesion to the substrate.

Important!

- Coat only NON-tissue culture treated polystyrene plasticware
- Use polypropylene tubes for dilution of StemAdhere
- Use a 5 ml glass pipette when passaging the cells

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Protocols for use of StemAdhere™

Materials required for coating:

- Non-tissue culture treated polystyrene plasticware (supplied 6-well plates or see page 5 for suitable alternatives)
- Sterile polypropylene conical tubes, 15 or 50 ml
- Sterile transfer pipettes
- Dilution Buffer (Product No. S2072-100ML)

Materials required for establishment and passaging of cells:

- Prewarmed culture medium (e.g. mTeSR®1, TeSR™2, or MEF-conditioned ES medium)
- Cell Release Buffer (Product No. S2117-200ML; acceptable results may also be obtained with Lonza Versene #17-711E)
- 5 ml glass pipette, Fisherbrand #13-678-27E

Coating plasticware with StemAdhere

Note: This protocol should be performed under aseptic conditions in a Biological Safety Cabinet. ***USE ONLY NON-TISSUE CULTURE TREATED PLASTICWARE****.*

1. Thaw the vial of StemAdhere at room temperature.
2. Dilute StemAdhere to 10 μg/ml with Dilution Buffer in a polypropylene conical tube. Prepare a sufficient volume for the desired plasticware (see Table 1 on page 4).
   The required dilution is 1:25, e.g. 240 μl StemAdhere diluted into 5.76 ml Dilution Buffer is sufficient for one 6-well plate. Optimal concentration of StemAdhere may vary depending upon cell type- try 15 and 20 μg/ml if attachment seems weak. Aliquot unused thawed StemAdhere in sterile tubes and refreeze at -80 °C for long term storage.
3. Mix gently (do not vortex).
4. Deposit required volume of diluted StemAdhere in a pool in the center of the well (e.g. 1 ml/well in a 6-well plate). After adding StemAdhere to the desired wells, gently shake the plate horizontally, side to side and forward-backward to spread the coating solution across the entire well or plate surface. Smaller volumes than recommended may be used, but will require more vigorous shaking to completely cover the surface.
5. Incubate 1 hr at 37 °C in a CO_2 incubator.
   After 37 °C incubation, plates may be stored overnight at 4 °C (wrap plates in Parafilm® or place in ziploc bag to minimize evaporation).
6. Remove StemAdhere solution and rinse once with Dilution Buffer before addition of cells. Do not allow wells to dry out.
7. Medium may be added and the plate held at 37 °C in a CO_2 incubator until addition of the cells (not more than 1 hr).

Converting cells from Matrigel to growth on StemAdhere™

It is essential to prevent proteolysis of cell surface proteins when preparing colonies of pluripotent cells for establishment on StemAdhere coated plates. The following non-enzymatic steps should be used for colonies growing on Matrigel or other feeder-free ECM substrates. Suggested volumes are for 6-well plates; adjust as needed.

1. Aspirate medium, rinse with 1 ml/well Cell Release Buffer and aspirate.
2. Add 1 ml/well Cell Release Buffer.
3. Incubate at room temperature for **9 minutes**.
4. Gently aspirate Cell Release Buffer.
5. Quickly remove the colonies from each well with 2 ml/well growth medium using a 5 ml glass pipette. Forcefully dispense the entire volume of medium with the pipette at a 90° angle to the plate, starting at the bottom of the well and moving upwards. The goal is to wash the entire content of the wells off the plate with one dispensing (or at most two) of the medium per well. **Avoid scraping.** The colonies should be easily removed without having to scrape the bottom of the plate. **Do not further break up the colonies**
6. Dropwise evenly distribute the colony suspension into each well of a medium containing prepared StemAdhere 6-well plate at desired split ratio (1:3 to 1:6 split recommended, depending on specific cell line)
   Optional: ROCK inhibitor and Thiazovivin have been reported to increase the efficiency of pluripotent cell survival after replating [3, 4]. ROCK inhibitor Y-27632 added at 10 μM final concentration, and Thiazovivin at 2 μM final concentration may enhance survival of cells and adaptation to StemAdhere.
7. Ensure that each well has at minimum 2 ml/well medium; add additional medium if needed.
8. Refeed colonies daily and monitor morphology and confluency. Passage when adjacent colonies begin to touch.

Converting feeder-dependent cells to growth on StemAdhere™

It is essential to prevent proteolysis of cell surface proteins when preparing feeder-dependent colonies of pluripotent cells for establishment on StemAdhere coated plates. The following manual passaging steps should be used for colonies growing on feeder cells.

1. Aspirate medium and replace with fresh 37 °C growth medium.
2. Manually cut each colony into 4-6 pieces using a sterile 26 g needle (attach to 1 ml syringe for easier handling). Alternatively, use a roller tool to cut the colonies, such as the StemPro® EZPassage™ disposable tool from Invitrogen.
3. Dislodge the colony fragments with a sterile pipette tip and aspirate the floating colonies with the culture medium.
4. Transfer to StemAdhere-coated well or dish of the same size. Do not dilute cells at this first passage (split ratio 1:1).
   Optional: ROCK inhibitor and Thiazovivin have been reported to increase the efficiency of pluripotent cell survival after replating [3, 4]. ROCK inhibitor Y-27632 added at 10 μM final concentration, and Thiazovivin at 2 μM final concentration may enhance survival of cells and adaptation to StemAdhere.

5. Refeed colonies daily and monitor morphology and confluency. Passage when adjacent colonies begin to touch.

Passaging cells grown on StemAdhere™

The split ratios of 1:3 to 1:6 from StemAdhere to StemAdhere have resulted in desireable attachment and confluence rates. It is recommended to use these ratios depending on the confluence of the starting wells. Suggested volumes are for 6-well plate, adjust as needed.

1. Aspirate medium from wells, rinse with 1ml/well Cell Release Buffer and aspirate.
2. Add 1 ml/well Cell Release Buffer.
3. Incubate at room temperature for 2-**3 minutes**.
4. Gently aspirate Cell Release Buffer.

9. Quickly remove the colonies from each well with 2 ml/well growth medium using a 5 ml glass pipette. Forcefully dispense the entire volume of medium with the pipette at a 90° angle to the plate, starting at the bottom of the well and moving upwards. The goal is to wash the entire content of the wells off the plate with one dispensing (or at most two) of the medium per well. **Avoid scraping.** The colonies should be easily removed without having to scrape the bottom of the plate. **Do not further break up the colonies**
5. Dropwise evenly distribute the colony suspension into each well of a medium containing prepared StemAdhere 6-well plate at desired split ratio (1:3 to 1:6 split recommended, depending on specific cell line)
   Optional: ROCK inhibitor and Thiazovivin have been reported to increase the efficiency of pluripotent cell survival after replating [3, 4]. ROCK inhibitor Y-27632 added at 10 μM final concentration, and Thiazovivin at 2 μM final concentration may enhance survival of cells and adaptation to StemAdhere.
6. Ensure that each well has at minimum 2 ml/well medium; add additional medium if needed.
7. Refeed colonies daily and monitor morphology and confluency. Passage when adjacent colonies begin to touch.

Table 1. Recommended volumes for coating

	Wells/dish				Dish/flask size			
	96	**24**	**12**	**6**	**60 mm**	**100 mm**	**T25**	**T75**
area per well (cm^2)	0.3	2	3.8	9.6	28.3	78.5	25	75
recommended volume per well (ml)	0.05	0.25	0.50	1.0	3.0	6.0	3.0	5.0
minimum volume per well (ml) at 78 µl/cm^2	0.02	0.16	0.30	0.75	2.20	6.12	1.95	5.85

Table 2. Polystyrene (non-TC treated) plasticware sources

Manufacturer	Type	Product No.	StemAdhere tested
Falcon/BD	24 well polystyrene	351147	Yes
Fisher	60 mm dish	08-757-BA	Yes
Greiner BioOne	6 well polystyrene	657185	Yes
	60 mm dish	628162	Yes

Available separately

StemAdhere Pluripotency Substrate	S2071-1MG
Cell Release Buffer (100 ml)	S2072-100ML
Dilution Buffer (100 ml)	S2117-100ML
6-well Polystyrene Plates (100/cs)	S2118-1CS

References

1. **Nagaoka, M., K. Si-Tayeb, T. Akaike, and S.A. Duncan, *Culture of human pluripotent stem cells using completely defined conditions on a recombinant E-cadherin substratum.* BMC Dev Biol. 10(1): p. 60.**
2. **Chen, T., D. Yuan, B. Wei, J. Jiang, J. Kang, K. Ling, Y. Gu, J. Li, L. Xiao, and G. Pei, *E-cadherin-Mediated Cell-Cell Contact is Critical for Induced Pluripotent Stem Cell Generation.* Stem Cells, 2010. 28(8):1315-25.**
3. **Watanabe, K., M. Ueno, D. Kamiya, A. Nishiyama, M. Matsumura, T. Wataya, J.B. Takahashi, S. Nishikawa, S. Nishikawa, K. Muguruma, and Y. Sasai, *A ROCK inhibitor permits survival of dissociated human embryonic stem cells.* Nat Biotechnol, 2007. 25(6): p. 681-6.**
4. **Lin, T., R. Ambasudhan, X. Yuan, W. Li, S. Hilcove, R. Abujarour, X. Lin, H.S. Hahm, E. Hao, A. Hayek, and S. Ding, *A chemical platform for improved induction of human iPSCs.* Nat Methods, 2009. 6(11): p. 805-8.**

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