CellAdhere™ Laminin-521

Matrix for maintenance of human ES and iPS cells in combination with mTeSR™1, mTeSR™ Plus, TeSR™-E8™, or TeSR™2

Catalog #77003	100 µg
77004	10 x 100 µg
200-0117	500 µg

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Product Description

CellAdhere™ Laminin-521 is a defined, xeno-free cell culture matrix that supports the long-term maintenance of human embryonic stem (ES) cells and induced pluripotent stem (iPS) cells in feeder-free conditions. Using CellAdhere™ Laminin-521 as a cell culture matrix increases single-cell attachment and survival compared to other matrices and does not require the addition of apoptotic inhibitors during plating (See Notes and Tips).[1-3] When used with mTeSR™1 (Catalog #85850), mTeSR™ Plus (Catalog #05825), TeSR™2 (Catalog #05860), or TeSR™-E8™ (Catalog #05990), the system allows complete control over the culture environment, resulting in consistent cell populations and reproducible results in downstream applications.

Properties

Storage: Store at -20°C.
Shelf Life: Stable until expiry date (EXP) on label.
Contains: A 100 µg/mL solution of recombinant protein composed of entirely human sequences.

Directions for Use

COATING CULTUREWARE WITH CELLADHERE™ LAMININ-521

Use sterile technique when coating cultureware with CellAdhere™ Laminin-521.

1. Thaw CellAdhere™ Laminin-521 at 2 - 8°C before use.

   NOTE: If not used immediately, store at 2 - 8°C for up to 3 months.

2. Dilute CellAdhere™ Laminin-521 in Dulbecco's phosphate-buffered saline with Ca++ and Mg++ to a final concentration of 5 - 10 µg/mL.

   NOTE: The required concentration of CellAdhere™ Laminin-521 can be cell-dependent and should be optimized for each application. When adapting to the new culture matrix, cells may benefit from a higher concentration of CellAdhere™ Laminin-521 for the first few passages.

3. Gently mix the diluted CellAdhere™ Laminin-521. Do not vortex.
4. Immediately add diluted CellAdhere™ Laminin-521 to cultureware. See Table 1 for recommended coating volumes.

Table 1. Recommended Volumes for Coating Cultureware with CellAdhere™ Laminin-521

CULTUREWARE	VOLUME OF DILUTED CellAdhere™ Laminin-521
12-well plate	0.5 mL/well
6-well plate	1 mL/well
T-25 cm^2 flask	3 mL/flask
T-75 cm^2 flask	8 mL/flask

5. Gently rock the cultureware back and forth to spread the CellAdhere™ Laminin-521 solution evenly across the entire surface.
6. Seal the cultureware to prevent evaporation of the CellAdhere™ Laminin-521 solution (e.g. with Parafilm®). Incubate at 2 - 8°C overnight. If a more rapid coating is required, incubate at 37°C for at least 2 hours before use.

   NOTE: If not used immediately, the cultureware can be stored at 2 - 8°C for up to 4 weeks after coating. Do not allow the culture surface to dry, as the matrix will become inactivated.

7. Aspirate CellAdhere™ Laminin-521 when cells are ready to be plated.

   NOTE: The coating does not require washing before use.

Notes and Tips

Although it is possible to passage human ES and iPS cells as single cells, this can result in selective pressure that could lead to genetic aberrations.[4-6] If passaging as single cells, check the karyotype frequently.

References

1. Lu HF et al. (2014) A defined xeno-free and feeder-free culture system for the derivation, expansion and direct differentiation of transgene-free patient-specific induced pluripotent stem cells. Biomaterials 35(9): 2816–26.
2. Rodin S et al. (2014) Clonal culturing of human embryonic stem cells on laminin-521/E-cadherin matrix in defined and xeno-free environment. Nat Commun 5: 3195.
3. Rodin S et al. (2014) Monolayer culturing and cloning of human pluripotent stem cells on laminin-521-based matrices under xeno-free and chemically defined conditions. Nat Protoc 9(10): 2354–68.
4. Draper JS et al. (2004) Recurrent gain of chromosomes 17q and 12 in cultured human embryonic stem cells. Nat Biotechnol. 22: 53–4.
5. The International Stem Cell Initiative. (2011) Screening ethnically diverse human embryonic stem cells identifies a chromosome 20 minimal amplicon conferring growth advantage. Nat Bio 29: 1132–44.
6. Garitaonandia I et al. (2015) Increased risk of genetic and epigenetic instability in human embryonic stem cells associated with specific culture conditions. PLoS ONE 10(2): e0118307.