Research use only

denovoMATRIX
ENABLING BIOLOGY IN VITRO

myMATRIX iPSC
BIOMATRICES FOR CELL CULTURE

User guide

myMATRIX iPSC is a xeno-free chemically defined matrix that combines a glycosaminoglycan and an extracellular matrix (ECM)-binding peptide supporting the long-term culture of human induced pluripotent stem cells (hiPSC).

STORAGE AND STABILITY

- myMATRIX iPSC can be stored at room temperature (RT).
- Upon opening, unused wells should be filled with a basal medium (eg. DMEM/F12, Advanced DMEM) and kept sterile for further use.
- Shelf life of the myMATRIX iPSC is 12 months. Please refer to product label for the exact expiration date of your product.

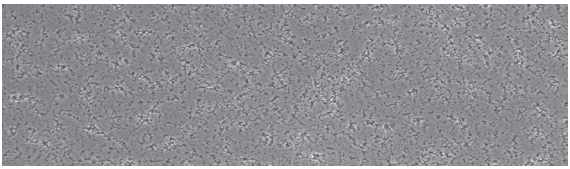

Figure 1. Microscopic appearance of myMATRIX iPSC.

PRODUCT FEATURES

- Sterile and defined, xeno-free matrices to support feeder-free culture of hiPSC
- Precoated plates that are ready-to-use
- Robust hiPSCs proliferation while maintaining pluripotency
- Can be used with a wide variety of media and reagents
- Optimized for clump and single-cell splitting routines

The following protocols are a general guideline on how to passage and maintain undifferentiated hiPSC on myMATRIX iPSC. Please follow specific guidelines of your media, cryopreservative, passaging reagent ect. provided by the manufacturer.
Please contact us for questions about your specific iPSC culture conditions.

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For more information about myMATRIX iPSC and our other products, please visit
www.denovomatrix.com

QUICK START – SUCCESSFUL CULTURE ON myMATRIX iPSC

Take your plate out of the packaging and fill the well with prewarmed medium.
Fill unused wells with a basal medium (e.g. DMEM/F12, Advanced DMEM) and leave the medium until use.
In order to successfully maintain undifferentiated iPSC check the cultures daily for areas of differentiation. Please note that in the initial days after splitting, colonies may appear unpacked and should not be mistaken for differentiated cells (Column A). When colonies show typical iPSC morphology (Column B), remove differentiated areas (Column C) e.g. by scraping. Cells are ready to split when the colonies are tightly packed. In addition, iPSC show a round morphology with large nuclei, notable nucleoli and a high nuclear-cytoplasmic ratio, as shown below. Change your medium daily or according to the media manufacturers instruction and your culture routine.

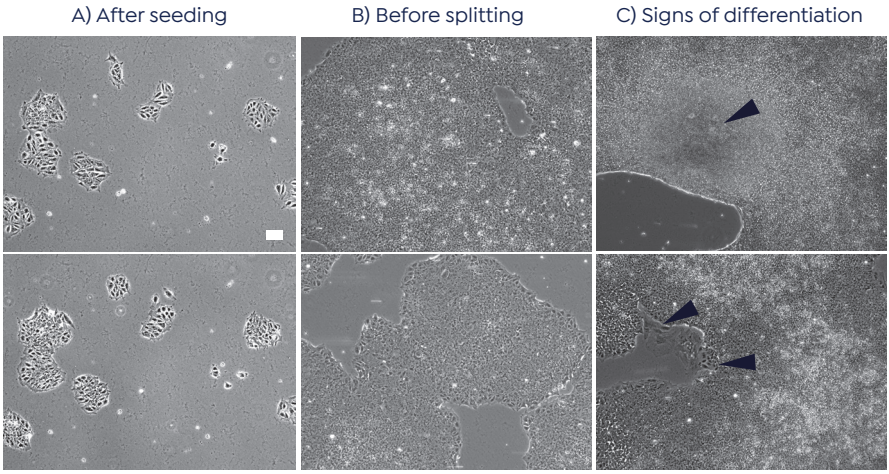

Figure 2. iPSC morphology overview. Human iPSC on myMATRIX iPSC A) after seeding, B) when they are ready to split (before splitting) and C) signs of differentiation. Scale bar 100 µm.

Key features and advices to keep in mind:

- Allow cells to gain a compact iPSC morphology before splitting.
- Remove differentiation at confluency.
- Matrix background may appear microscopically as small structures at high magnification (see first page).

GUIDE TO myMATRIX iPSC

The following exemplifies how you seed in iPSCs from a frozen stock, maintain them in an undifferentiated state and subsequently passage them with an clump- or single-cell split. Please refer to the specific guidelines of your medium manufacturer concerning stability, handling and preparation of the medium.

ADAPTATION TO myMATRIX iPSC

We recommend to adapt your cells by routine clump-seeding (see also enzyme-free passage, page 5) from ongoing proliferating cultures from other feeder-free cultures onto the myMATRIX iPSC using a method of choice. However, it is also possible to directly seed your cryopreserved iPSCs onto the myMATRIX iPSC after thawing (see instructions below). In that case, please make sure that you have more cells cryopreserved as a backup.

Thawing cells to start culture

- Take your plate out of the cover and fill the well with prewarmed medium supplemented with 10 µM ROCK inhibitor (ROCKi).
- Fill unused wells with a basal medium (e.g. DMEM/F12, Advanced DMEM). Leave the medium inside the well until use.
- Thaw cells quickly at 37°C and add 1 mL medium to take up cells from freezing vial.
- Pipette cells slowly, drop-wise into 5 mL pre-warmed medium + 10 µM ROCKi in a 15 mL tube.
- Centrifuge cells for 3 min at 250 x g to remove your cryopreservative.
- Aspirate the supernatant, then carefully resuspend the cell pellet by gently pipetting 1 mL medium up and down.
- Count the number of viable cells and seed 200.000 cells directly onto a 6-well containing 2 mL of medium.
- After 24 hours replace ROCK inhibitor-containing medium with fresh medium without ROCKi.

Growing to confluency

- Routinely check your cells for growth and signs of differentiation (Fig. 2). Monitor the morphology over time after splitting (Fig. 3 A–C).
- Replace the medium every 24 h with 1.5–2 mL of fresh medium equilibrated to RT.
- Do not touch the cells or the surface while removing the used medium and pipette the fresh medium into the well by slowly letting the medium run down the wall of the well.
- The cells are ready for splitting when they have developed a typical iPSC morphology (Fig. 2, Fig. 3C)
- Note: Do not split the iPSCs when the majority of colonies still show gaps within the colonies (Fig. 3B).

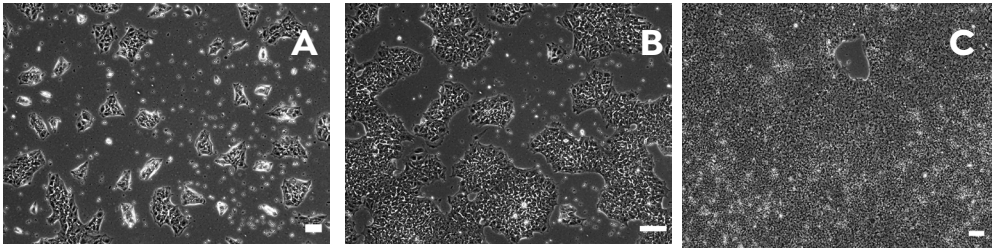

Figure 3. Human iPSC on myMATRIX iPSC growing cofluent. Shown are cells grown for (A) 4 days, (B) 5 days (not ready for splitting) and (C) 6 days post-seeding (ready for splitting). Individual cells show a round morphology with large, notable nucleoli and a high nuclear-cytoplasmic ratio. At this time, cells are ready for splitting and signs of differentiation can be removed manually. Scale bar: 100 µm.

PASSAGE OF iPSC FROM myMATRIX iPSC

We recommend to split iPSCs in clumps using enzyme-free passaging reagents. However, myMATRIX iPSC is also optimized to support enzymatic passaging reagents for clump- or single-cell splitting. For the individual splitting methods, please follow the manufacturers' protocols. Below you can find some additional advice that will help you to specifically passage on myMATRIX iPSC.

Enzyme-free passaging

- Remove areas of differentiation only when cells are confluent and compact (Fig. 4A).
- Optimize the incubation time with your passaging reagent (Fig. 4B).
- Your clumps can be diluted at a ratio of 1:5-1:40 with a mean size of approximately 50-200 µm (Fig. 4C).
- If colonies do not detach using pipetting, take out your medium containing the detached clumps and use new medium to detach the remaining colonies. Repeat this procedure until all colonies are detached or alternatively scrape the cells off.
- Within the first 24 hours after seeding, cells should be minimally disturbed.

Enzymatic passaging

- Remove areas of differentiation only when cells are confluent and compact.
- If cells do not detach using pipetting you may scrape the cells from the plate.
- The medium during single-cell seeding on myMATRIX iPSC can be supplemented with Rho-associated protein kinase (ROCK) inhibitor.
- Within the first 24 hours after seeding, cells should be minimally disturbed.

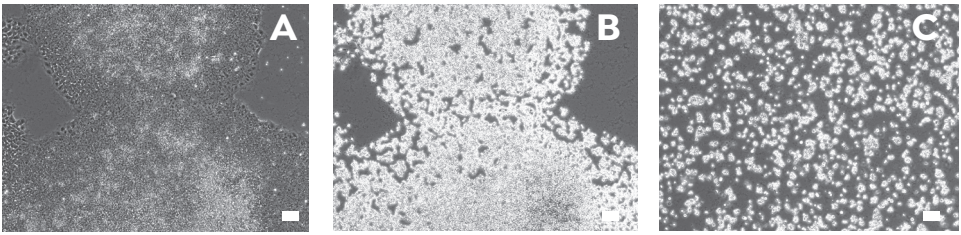

Figure 4. Time course of a clump-split on myMATRIX iPSC. A) Colonies are ready-to-split when they show typical iPSC morphology; B) iPSCs treated with an enzyme-free splitting reagent and ready to be sized into clumps; C) Medium-sized iPSC clumps. Scale bar 100 µm.

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