Alternative Media and Media Components for growing KOLF2.1J and derived iPSC lines

Updated April 15, 2023

Please adhere to the JAX standard protocol online when thawing JAX iPSC lines. We recommend expanding and freezing down a couple of vials for each line before changing to alternative growth conditions.

If you cannot obtain the recommended products and need to consider other media components, the below alternative options have proven to work well with JAX iPSCs.

Coating:

- **Recommended: Synthemax® II-SC Substrate (Corning)**. Synthetic matrix, very uniform. iPS cells attach very strongly.
- **Alternative I: Vitronectin substrate.** Very uniform morphology.
- **Alternative II: Matrigel® (Corning).** Made from animal material and could therefore result in slightly more batch-to-batch variability. iPS cells appear more elongated when using this substrate.

iPS cell lines can be thawed and grown on all of the above. Differences in morphology may be observed dependent upon the coating matrix used, however, this will not affect iPS cell function.

Media:

- **Recommended: StemFlex™ (Gibco Life Technologies).** Best at keeping iPS cell lines in an undifferentiated state (almost 100% on vitronectin substrate). We recommend use when thawing and for every other day feeding. We don't advise to use just prior to differentiating cells.
- **Alternative I: StemMACS™ iPS-Brew XF, human (Miltenyi Biotec).** Slightly faster growth rate than StemFlex. Media color change not as apparent. Not recommended for single cell cloning.
- **Alternative II: mTeSR™ Plus**. Similar to StemFlex but some minor spontaneous differentiation visible (within average range). Used for every other day feeding but one might want to consider every day feeding when cells are approaching confluence.

ROCK inhibitors:

- **Recommended: RevitaCell™ Supplement** (Cat# A26445-01, Gibco Life Technologies)
- **Y-compound, CloneR™, or CloneR™2** (STEMCELL Technologies), **Chroman 1** (Tocris)**:** All work well for thawing cells. Leave for one day when thawing cells.
- **CloneR™** products work very well. With the newest CloneR™2, we observe near 100% cell survival. Leave in media for 2 days to obtain best results.

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- ROCKi should always be used when thawing cryo-preserved cells or when iPS cells are split as single cells (e.g., when using Accutase) rather than clumps, as these compounds promote survival of iPS cell lines when in single cell state. We recommend using ROCKi when cells are plated as single cells for certain applications (e.g., isolation of single cell-derived clones)
- No need to add ROCKi when cells are split and plated as clumps. We recommend routine passaging of iPS cells as clumps

Freezing Media:

- **Recommended: Knockout™ Serum Replacement (Gibco Life Technologies) + 10% DMSO.** Prepared fresh and filter sterilized. Vials of cells are placed directly in a -80°C freezer overnight before transferring to liquid Nitrogen. Slow cooling is not required using this freezing media.

Please note that many of those reagents have a short shelf-life, therefore, check expiry dates before using any of those media components.