

# PRODUCT INFORMATION

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## GS2-M®

**Catalog Number:** Y40030

**Size:** GS2-M consists of 2 components which must be combined prior to use:

- 100 mL media
- 100  $\mu$ L 2i inhibitor supplement (NOTE: Supplement contains DMSO)

**Applications:** Efficient long-term maintenance of both mouse and human embryonic and induced pluripotent stem cells (ES and iPS, respectively) in serum-free culture conditions.

**Description:** GS2-M is a proprietary, defined and serum-free cell culture media formulation for the generation of mouse ES and iPS cell lines in the presence of two selective small molecule inhibitors CHIR99021 and PD0325901 ('2i') that act to eliminate differentiation-inducing signals from GSK3  $\beta$  and ERK/MEK, respectively, and promote cell survival. LIF can also be added to orchestrate the efficient conversion of partial or pre-iPS mouse cells into fully pluripotent iPS cells via Nanog<sup>1, 2</sup>. Long term maintenance of these naïve, 'ground state' mouse pluripotent stem cells is then solely dependent on GS2-M alone.

Recently, the 2i formulation has also been used for non-germ line competent or recalcitrant mouse ES cells derivation or conversion into complete germ line potency<sup>3</sup>, and to generate naïve human and livestock species iPS cells<sup>4, 5</sup>.

**Storage:** Upon receipt, store the media at -20°C and the GS2-M 2i inhibitor supplement at -80°C until ready to use. When stored under these conditions, the products are stable for 12 months from the date of manufacture (see label). Once thawed and combined, store at 4°C and use within 2 weeks.

**This product is light sensitive, and should be protected from light.**

**Preparation:** Thaw the medium in a water bath (37°C) in the dark, and remove it from the water bath just before the medium has completely thawed (i.e., do not allow the media to warm up). Then, mix the medium gently and thaw completely. Alternatively, thaw the medium at 4°C while protecting from light. If a precipitate appears in the medium, leave it at 4°C overnight to completely dissolve the precipitate. Do not use media with visible precipitate; ensure it is dissolved before use.

Thaw the 100  $\mu$ L GS2-M 2i supplement at room temperature (for no longer than 5 minutes), and microfuge immediately. Add aseptically to warmed medium and mix thoroughly to ensure the supplement is completely distributed.

For the mouse iPS cell **re-programming phase or clonal cell seeding only**, add 10 ng/mL LIF to the complete GS2-M 2i medium. Do not filter sterilize.

**Additional Reagents Required:**

**A. For mouse ES and iPS cells.**

Culture vessels are pre-coated with either 0.1% gelatin type A in PBS solution (at room temperature for 15 minutes or more), or precoated sequentially with 0.01% poly-L-ornithine hydrobromide (at 37°C for 30 minutes or more), washing twice with PBS, followed by 10  $\mu$ g/mL laminin in PBS (at 37°C for 3 hours or more).

**B. For human iPS cells.**

Please refer to Wang W, *et al.* (2011) reference for more detailed reagent and culture requirements to generate and propagate 'naïve' human iPS cells<sup>4</sup>.

**Quality Control:**

SC Proven™ media products undergo rigorous quality control procedures before release.

**References:**

1. Silva J, *et al.* Promotion of reprogramming to ground state pluripotency by signal inhibition. *PLoS Biol.* (2008) **6**: e253.
2. Silva J, *et al.* Nanog is the gateway to the pluripotent ground state. *Cell.* (2009) **138**(4): 722-737.
3. Nichols J, *et al.* Validated germline-competent embryonic stem cell lines from non-obese diabetic mice. *Nat Med.* (2009) **15**: 814-818.
4. Wang W, *et al.* Rapid and efficient reprogramming of somatic cells to induced pluripotent stem cells by retinoic acid receptor gamma and liver receptor homolog. *PNAS (USA)*. (2011) **108**(45): 18283-18288.
5. Reviewed in: Malaver-Ortega LF, *et al.* *Theriogenology*. (2012) **78**(8): 1749-1762.

**Note**

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