Research

StemSure® –

Specialist Stem Cell Culture Reagents

Culturing stem cells in an undifferentiated state can require complex culture conditions. Historically a growth-inactivated feeder-cell layer was used, which provided a sticky surface to which cells could attach and also released nutrients into the culture medium. A number of measures can now be taken to eliminate the need for a feeder-cell layer or serum supplemented media, such as gelatin coated culture surfaces and serum replacements.

The StemSure® range has been specifically designed for use in stem cell culture protocols to give optimal results. Every batch of StemSure reagent is subject to rigorous quality assurance checks including:

- Mouse ES D3/ human iPS 201B7 cell culture
- Colony formation and cell proliferation
- Alkaline phosphatase staining
- Sterility test
- Mycoplasma testing

For mouse ES (MES) cell culture Wako offer StemSure D-MEM, a high glucose formulation with phenol red and sodium pyruvate that has been optimised for the culture of mouse ES cells. This formulation has a lower osmotic pressure than standard D-MEM media to make it suitable for ES culture. Use together with StemSure Serum Replacement and StemSure LIF for culture of undifferentiated Mouse ES D3 cells and other MES lines, (Figure 1). Alternatively, for human ES or iPS culture protocols use Wako's StemSure hPSC Medium, intended for feeder-cell and serum free culture. This formulation is low in protein and does not contain albumin (Figure 2).

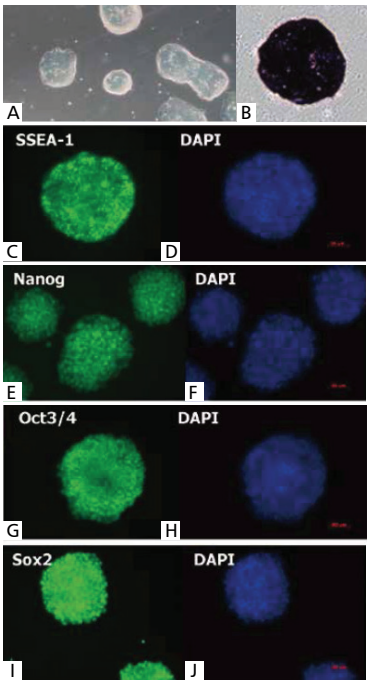

Figure 1. Mouse ES D3 cells cultured in StemSure D-MEM +15% SSR +2mmol/L L-Glutamine + 1xMEM Non-essential Amino acids + 0.1mmol/L StemSure 2 Mercaptoethanol (2 ME) + 1x penicillin-streptomycin + 1000 U/ml StemSure LIF in 12 well collagen coated plates. (A) Cell morphology under bright phase was consistent with an undifferentiated state supported by (B) positive alkaline phosphatase (ALP) staining and expression of (C) stage-specific embryonic antigen-1 (SSEA-1) and transcription factors (E) NANOG, (G) OCT3/4 and (I) SOX2 with (D,F,H,J) DAPI nuclear stain for comparison.

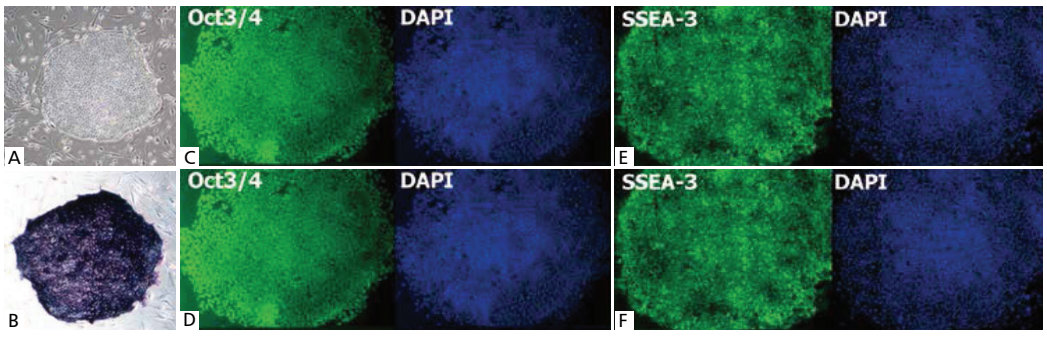

Figure 2. Human iPS 201B7 line cultured in D-MEM/Ham's F-12 + 20% StemSure SSR + 2 mmol/L L-Glutamine + 1xMEM Non-essential Amino Acids + 0.1 mmol/L StemSure 2ME + 1 x Pen-strep + 5ng/ml bFGF. Undifferentiated state was maintained as indicated by cell morphology (A), ALP staining (B) and expression of undifferentiation markers Oct3/4, Sox2, SSEA-3 and Tra-1-81(C-F) with DAPI nuclear stain for comparison.

Not for diagnostic use

Also available in the StemSure range are Monothioglycerol (MTG) and 2 Mercaptoethanol (2 ME), reducing agents commonly added to culture media to prevent build up of toxic oxygen radicals which can be harmful to ES and iPS cells.

MTG and 2ME can be used alone or in combination to suit the requirement of your particular culture model – whether maintaining undifferentiated cells or programming for lineage-specific differentiation.

Cryopreservation of stem cells can be problematic, ES cells tend to be more fragile and vulnerable to differentiation in cold stress which can lead to poor recovery and survival when thawed. When it comes to storing your precious cell stocks Wako also offer StemSure Freezing media. Mouse ES D3 cells have been shown to retain undifferentiated cell morphology and marker expression after preservation with the StemSure formula (Figure 3).

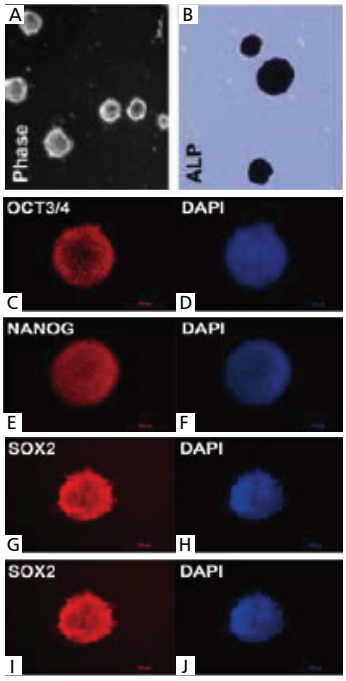

Figure 3. Mouse ES D3 cells (1-2x10^6) suspended in 1ml StemSure Freezing Medium before cryopreservation <-80°C. Cells subsequently defrosted at 37°C in water bath and returned to culture. (A) Cell morphology, (B) alkaline phosphatase and markers of undifferentiation (C) OCT3/4, (E) Nanog, (G) SOX2, (I) and SSEA-1 with (D,F,H,J) DAPI nuclear stain for comparison.

StemSure Culture Reagents

Product Code	Description	Pack Size
197-16275	StemSure® D-MEM	500ml
197-16775	StemSure Serum Replacement	500ml
197-17571	StemSure hPSC Medium	100ml
198-15781	StemSure 10mmol/l 2-Mercaptoethanol S	100ml
195-15791	StemSure 50mmol/l Monothioglycerol	100ml
195-16031	StemSure Freezing Medium	100ml

Associated products for ES & iPS culture

ES/iPS Culture Reagents

Product Code	Description	Pack Size
039-24111	CultureSure® A-83-01	2mg
035-24113	CultureSure® A-83-01	10mg
029-16241	6-Bromoindirubin-3'-oxime	1mg
038-24681	CultureSure® 10mmol/l CHIR99021 in DMSO	300µl
034-23103	CHIR99021	5mg
169-19211	PD-98059	5mg
165-26761	PD184352	5mg
199-16551	SB203580	1mg
193-16733	SU5402	5mg
211-01051	U0126	5mg
129-05601	LIF, Human, recombinant solution	1ml
068-05384	Fibroblast Growth Factor (basic) Human (bFGF)	100µg
034-24024	Y-27632 CultureSure	25mg

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