

AdvanceSTEM Serum Replacement

HYCLONE MEDIA AND SUPPLEMENTS

HyClone™ AdvanceSTEM™ Serum Replacement has been developed to support the growth of undifferentiated pluripotent embryonic stem cells in culture with no requirement for fetal bovine serum (FBS) supplementation (Fig 1). The product is intended for use with HyClone AdvanceSTEM Low Osmo DMEM.

Key features of AdvanceSTEM Serum Replacement include:

- Designed to maintain mESCs in an undifferentiated state
- Serum-free formulation
- Intended for use with HyClone AdvanceSTEM Low Osmo DMEM

Specifications

AdvanceSTEM Serum Replacement is a serum-free formulation intended for use with HyClone AdvanceSTEM Low Osmo DMEM to maintain murine embryonic stem cells (mESCs) in an undifferentiated state. Concentrations of 15% to 20% AdvanceSTEM Serum Replacement supplementation are suggested. Do not use at concentrations $\geq 30\%$. The product does not contain leukemia inhibitory factor (LIF). Supplementation with LIF is required for murine embryonic stem cell culture. AdvanceSTEM Serum Replacement is not for plating mouse embryonic feeder (MEF) cells.

Figure 2 shows mESCs, derived and propagated in AdvanceSTEM Low Osmo DMEM and AdvanceSTEM Serum Replacement, growing on MEF cells.

Suggested preparation

Preparation of 200 mL AdvanceSTEM Low Osmo DMEM supplemented with 20% AdvanceSTEM Serum Replacement is given in Table 1. Store prepared medium at 2°C to 8°C and discard any unused medium after two weeks.



Fig 1. AdvanceSTEM Serum Replacement supports the growth of undifferentiated pluripotent embryonic stem cells in culture.

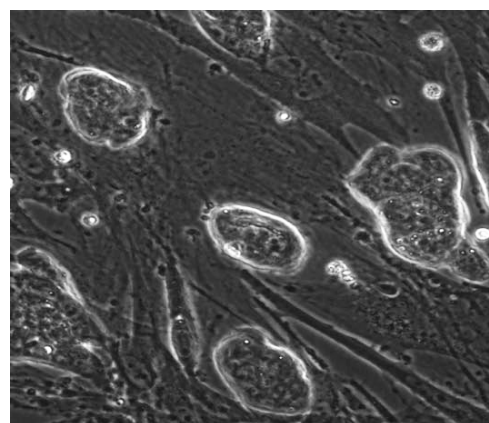


Fig 2. C57BL/6J mESCs growing on MEF cells. The mESCs were derived and propagated in AdvanceSTEM Low Osmo DMEM supplemented with AdvanceSTEM Serum Replacement. Photo courtesy of Primogenix, Inc.

Table 1. Preparation of 200 mL AdvanceSTEM Low Osmo DMEM supplemented with 20% AdvanceSTEM Serum Replacement

Brand	Amount for 200 mL	Product	Product code
HyClone	158 mL	AdvanceSTEM Low Osmo DMEM	SH30870.01
HyClone	40 mL	AdvanceSTEM Serum Replacement	SH30874.01, SH30874.02
HyClone	2.0 mL	HyClone AdvanceSTEM ES Qualified L-glutamine 200 mM	SH30852.01
Millipore	20 µL	ESGRO™ mLIF supplement	ESG1107

General culture recommendations

- Cultures should be incubated at 37°C in a 5% CO₂ environment.
- To maintain mESCs in an undifferentiated state, examine cells daily and passage if necessary. Passage cells before signs of differentiation appear. Typically cells are passaged every two days.
 - 15% to 20% serum replacement supplementation.
 - Leukemia inhibitor factor (LIF) supplementation 1000 U/mL is recommended for mESC culture.
- Change medium daily (follow Table 1 for AdvanceSTEM Serum Replacement supplementation).
- Trypsinize and passage cells every other day at approximately a ratio of 1 to 5 in a new flask of MEF cells. Roughly 1/5 of the trypsinized cells are passaged to a new flask of inactivated MEF cells; the remaining cells are either frozen or used experimentally as needed.

Trypsinization using serum replacement supplementation protocols:

As this medium does not contain fetal bovine serum (FBS), trypsin should be inactivated or removed.

- Aspirate medium.
- Rinse flask with Dulbecco's PBS (without Mg²⁺, without Ca²⁺).
- Add 0.25% trypsin/EDTA to cover the surface of the culture vessel.
- Place vessel into the incubator for 3 to 5 min, observing every few minutes until cells begin lifting from the culture vessel. If cells have not yet lifted, gently shake the vessel to cover the cells with trypsin and return vessel to the incubator.
- When cells have lifted from the vessel (less than 5 min total time), mix trypsinized cells with 2× volume of MEF medium (or DMEM supplemented with 15% FBS).

- Spin down the cells at 1000 rpm for 5 min.
- Aspirate the MEF medium and add growth medium (Table 1).
- Resuspend cell pellet.
- Plate in AdvanceSTEM Low Osmo DMEM supplemented with AdvanceSTEM Serum Replacement (Table 1) on a healthy layer of inactivated MEF cells.

Quality control testing

Quality control test specifications are listed in Table 2.

Table 2. Test specifications¹

Appearance	Clear yellowish liquid
Osmolality	For information only
pH	For information only
Sterility	No Growth (bacteria or fungi)
Endotoxin	≤ 10 EU/mL
Application	Plating efficiency of mESCs ¹

¹ Refer to certificate of analysis for actual results.

Related products

Classic culture conditions for mESCs include co-culturing with primary MEF cells in medium containing FBS and supplements. The following products are used in the culture of mESCs.

HyClone ES Screened FBS

For research involving embryonic stem cells (ESCs), it is critical to maintain the cells in their undifferentiated state. ES Screened FBS has been screened for the ability to promote the rapid growth of ES cells while retaining their pluripotent state, eliminating the need for customers to prescreen serum lots.

ES Qualified Stem Cell Products

HyClone AdvanceSTEM line of stem cell culture products has been developed specifically for applications in stem cell research. The line includes numerous products designed to support a wide range of applications, from the growth and maintenance of murine embryonic and human adult somatic stem cells to the directed differentiation of mesenchymal stem cells into adipocytes, chondrocytes and osteocytes. In addition to AdvanceSTEM Serum Replacement, the range of products includes:

- AdvanceSTEM Low Osmo DMEM
- AdvanceSTEM ES Qualified L-glutamine
- AdvanceSTEM ES Qualified Non-Essential Amino Acids
- AdvanceSTEM ES Qualified HEPES
- AdvanceSTEM ES Qualified PBS
- AdvanceSTEM ES Qualified Dulbecco's Phosphate Buffered Saline (DPBS)
- AdvanceSTEM IMDM4SC without L-glutamine
- AdvanceSTEM DMEM4SC with 4.5 mg/L Glucose, without L-glutamine and Sodium Pyruvate

Ordering information

Product	Size	Product code
HyClone AdvanceSTEM Serum Replacement, Frozen	50 mL	SH30874.01
	100 mL	SH30874.02
	500 mL	SH30874.03
Related products	Size	Product code
HyClone AdvanceSTEM Low Osmo DMEM Without L-glutamine	500 mL	SH30870.01
	1000 mL	SH30870.02
HyClone ES Screened FBS	500 mL	SH30070.03E
HyClone AdvanceSTEM ES Qualified L-glutamine	100 mL	SH30852.01
HyClone AdvanceSTEM ES Qualified Non-Essential Amino Acids	100 mL	SH30853.01
HyClone AdvanceSTEM ES Qualified HEPES	100 mL	SH30851.01
HyClone AdvanceSTEM ES Qualified PBS	1000 mL	SH30850.03
	500 mL	SH30850.02
AdvanceSTEM ES Qualified Dulbecco's Phosphate Buffered Saline (DPBS)	1000 mL	SH30850.03
	500 mL	SH30822.01
AdvanceSTEM IMDM4SC Without L-glutamine	1000 mL	SH30822.02
	500 mL	SH30824.01
AdvanceSTEM DMEM4SC With 4.5 mg/L glucose Without L-glutamine and sodium pyruvate	1000 mL	SH30824.04

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