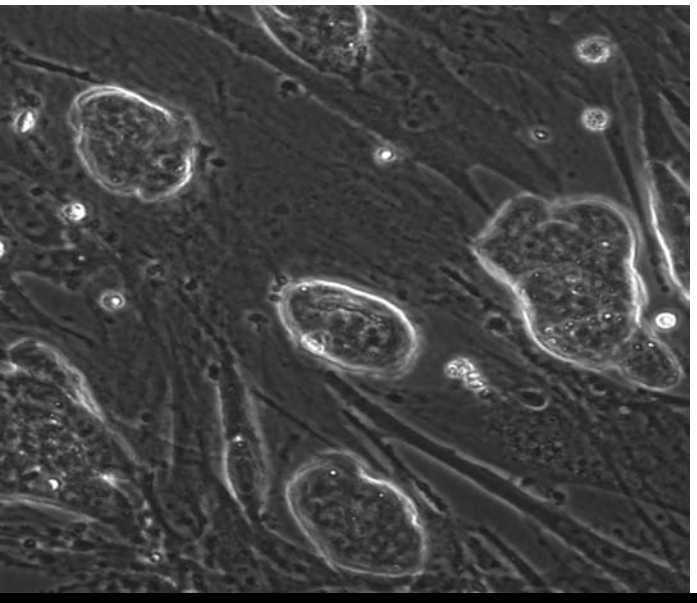
Product Insert

The Thermo Scientific HyClone AdvanceSTEM™ Serum Replacement product has been developed to support the growth of undifferentiated pluripotent embryonic stem cells.

Thermo Scientific HyClone AdvanceSTEM™ Serum Replacement

Features

- Designed to maintain murine embryonic stem cells (mESC) in an undifferentiated state.
- Not recommended for plating mouse embryonic feeder cells (MEFs).
- Formulation does not contain serum.
- Does not contain leukemia inhibitory factory (LIF); supplementation with LIF is required for murine embryonic stem cell culture.
- Concentrations of 15 to 20% Serum Replacement supplementation are suggested. Do not use at concentrations ≥30%.
- Designed for use with HyClone AdvanceSTEM™ Low Osmo DMEM.



Instructions for Use:

1. Cultures should be incubated at 37ºC and in a 5% CO_2 environment.
2. To maintain mESC 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.
   a. 15 – 20% serum replacement supplementation
   b. Leukemia inhibitor factor (LIF) supplementation 1000U/ml) is recommended for mESC culture
3. Change medium daily (follow TABLE 2 outlined in this product insert for Thermo Scientific HyClone AdvanceSTEM™ Serum Replacement supplementation).
4. Trypsinize and passage cells every other day at approximately a 1-to-5 ratio in a new flask of mouse embryonic feeders (MEFs). Roughly 1/5 of the trypsinized cells are passaged to a new flask of inactivated feeder cells, the remaining cells are either frozen or used experimentally as needed.
   a. Trypsinization using serum replacement supplementation protocols:
   Since this medium does not contain fetal bovine serum (FBS), trypsin should be inactivated and/or removed.
   i. Aspirate medium
   ii. Rinse flask with Dulbecco's- PBS (without Mg++, without Ca++)
   iii. Add 0.25% trypsin/EDTA to cover the surface of the culture vessel.
   iv. Place vessel into the incubator for 3-5 minutes, 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.
   v. When cells have lifted from the vessel (less than 5 minutes total time), mix trypsinized cells with 2X volume of MEF medium (or DMEM supplemented with 15% FBS).
   vi. Spin down the cells, 1000rpm for 5 minutes.
   vii. Aspirate the MEF medium, and add growth medium (TABLE 2)
   viii. Re-suspend cell pellet.
   ix. Plate in Thermo Scientific HyClone AdvanceSTEM™ Low Osmo DMEM supplemented with HyClone AdvanceSTEM™ Serum Replacement (TABLE 2) on a healthy layer of inactivated mouse embryonic feeder cells (MEFs).

Figure 1:
C57BL/6J mESCs growing on MEF feeder cells. These mESCs were derived and propagated in HyClone AdvanceSTEM™ Low Osmo DMEM and AdvanceSTEM Serum Replacement.
Photo courtesy of Primogenix, Inc.

Table 1:
QC Testing

Test	Specification*
Appearance	Clear straw colored liquid
Osmolality	For information only
pH	For information only
Sterility	No Growth (Bacteria or Fungi)
Endotoxin	≤10 EU/ml
Application	Plating efficiency of mESC*

***Refer to certificate of analysis for results.**

Table 2:
Preparation of 200 mL AdvanceSTEM™ Low Osmo DMEM supplemented with 20% AdvanceSTEM™ Serum Replacement

Brand	Amount for 200 mL	Product	Part Number
Thermo Scientific	158 mL	HyClone AdvanceSTEM™ Low Osmo DMEM	SH30870.01 or SH30870.02
Thermo Scientific	40 mL	HyClone AdvanceSTEM™ Serum Replacement	SH30874.01 or SH30874.02
Thermo Scientific	2.0 mL	HyClone AdvanceSTEM™ ES Qualified L-glutamine 200 mM Solution	SH30852.01
Millipore/ Chemicon	20 ul	ESGRO-Mouse LIF	ESG1107

Store at 2-8ºC and discard any unused medium after 2 weeks.

Ordering Information

Name	Part Number	Unit Size	Description
HyClone AdvanceSTEM™ Serum Replacement	SH30874.01 SH30874.02	500 mL 1000 mL	Our serum replacement has been developed to support the growth of undifferentiated embryonic stem cells in culture with no requirement for fetal bovine serum supplementation.

Related Products

Classic culture conditions for murine embryonic stem cells (mESCs) include culturing in medium containing FBS and supplements, which are then co-cultured with primary mouse embryonic fibroblasts (MEFs). The following are several products used in the culture of mESCs.

HyClone ES Screened FBS	SH30070.03E	500 mL
HyClone AdvanceSTEM™ ES Qualified L-glutamine	SH30852.01	100 mL
HyClone AdvanceSTEM™ ES Qualified Non-Essential Amino Acids	SH30853.01	100 mL
HyClone AdvanceSTEM™ ES Qualified HEPES	SH30851.01	100 mL
HyClone AdvanceSTEM™ ES Qualified PBS	SH30850.03	1000 mL

HyClone - Media•BPC•Sera
925 West 1800 South
Logan, UT
84321

In Americas/Asia
435-792-8000
435-792-8001 fax

In Europe
+32 53 85 71 80
+32 53 85 74 31 fax

www.thermo.com/hyclone

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