

Thermo Scientific HyClone AdvanceSTEM™ Low Osmo DMEM

The Thermo Scientific HyClone AdvanceSTEM™ Low Osmo DMEM product has an optimized osmolality (osmo) which approximates that of murine embryonic tissue. We've developed the formulation specifically to support the growth and maintenance of murine embryonic stem cells.

Features

- Formulated to more closely approximate the osmolality of embryonic tissues
- Specifically formulated to maintain physiological pH at 37°C and 5% CO₂
- Contains HEPES, β-mercaptoethanol and non-essential amino acids (NEAA).
- Formulation does not contain L-glutamine to allow users to supplement at the L-glutamine concentration of their choice.
- This basal medium requires supplementation with fetal bovine serum (FBS) or serum replacement. Recommended supplementation is 15% FBS or 20% Thermo Scientific HyClone AdvanceSTEM Serum Replacement.
- Does not contain leukemia inhibitory factor (LIF); supplementation with LIF is required with this formulation when FBS or serum replacements are used for the culture of mESCs.

Instructions for Use:

1. Cultures should be incubated at 37°C and in a 5% CO₂ environment.
2. To maintain mESC in an undifferentiated state, examine cells daily under a phase contrast microscope and passage if necessary. Passage cells before signs of differentiation appear. It is typical for cells to be passaged every two to three days depending on the line.
3. Change medium daily use medium supplemented as follows:
 - a. 20% AdvanceSTEM Serum Replacement supplementation (TABLE 2), or 15% FBS supplementation (TABLE 3)
 - b. Leukemia inhibitory factor (LIF) supplementation (1000U/ml) is required
 - c. L-glutamine supplementation is required
4. Trypsinize and passage cells every other day at approximately a 1-to-5 ratio in a new flask of inactivated mouse embryonic feeders, MEFs (1/5 of the trypsinized cells are replated) as needed. Please note: some mESC lines may proliferate more quickly in this rich medium than other basal media. Monitor closely and subculture as needed.
 - a. When using FBS, follow trypsinization methods outlined in Thermo Scientific SC Protocols Sheet 0005 for Murine Embryonic Stem Cell.
 - B. When using Serum Replacement, follow trypsinization methods outlined in the AdvanceSTEM™ Serum Replacement product insert – Instructions for use section.

Note: Subculturing mESC by trypsinization is a proven method for dissociation of mESC colonies; however, care should be taken when passaging in medium containing serum replacements, as they do not contain the trypsin inhibitors found in FBS making it necessary to remove the trypsin before plating the cells.

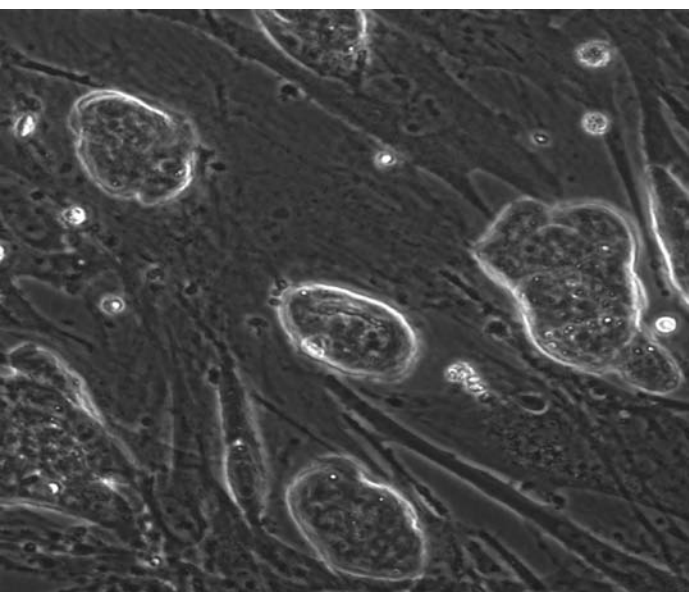
Table 1:
QC Testing

Test	Specification*
Appearance	Clear Reddish solution
Osmolality	270-290 mOsm/kg
pH	6.9-7.3
Sterility	No Growth (Bacteria or Fungi)
Endotoxin	≤1 EU/ml
Application	Plating efficiency of mESC*

*Refer to certificate of analysis for results.

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.



Media Supplementation Formulas

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 2-8°C and discard any unused medium after 2 weeks.

Table 3:

Preparation of 200 mL AdvanceSTEM™ Low Osmo DMEM supplemented with 15% FBS

Brand	Amount for 200 mL	Product	Part Number
Thermo Scientific	168 mL	HyClone AdvanceSTEM™ Low Osmo DMEM	SH30870.01 or SH30870.02
Thermo Scientific	30 mL	HyClone FBS-ES Qualified	SH30070.03E
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 2-8°C and discard any unused medium after 2 weeks.

Ordering Information

Name	Part Number	Unit Size
HyClone AdvanceSTEM™ Low Osmo DMEM without L-glutamine	SH30870.01	500 mL
	SH30870.02	1000 mL
Related Products		
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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