Hybridoma Cell Culture Media

PromoCell

Instruction Manual

Product	Size	Catalog Number
Hybridoma Growth Medium 6 Express	500 ml	C-78410
Hybridoma Growth Medium 6 Express, phenol red-free	500 ml	C-78417
Hybridoma Growth Medium 7 Express	500 ml	C-78440
Hybridoma Growth Medium 7 Express, phenol red-free	500 ml	C-78447

Description

Hybridoma Growth Media (HYGM) 6 and 7 are proprietary, fully defined media that can be used for the cultivation of various different hybridomas/cell lines. HYGM-6 is serum-free, HYGM-7 is protein-free. Both media are available with or without phenol red to prevent interference of the dye with chromogenic assays.

HYGM-Media are ready-to-use.

Instructions For Use

Physical Conditions

Use media at 37°C +/- 0.5°C in a humidified atmosphere of 5-8% CO_2. Caps of flasks should be loosened to permit gas exchange. Cultures may be grown in stationary suspension culture (e.g., T-flask) or in agitated suspension culture (shaker or spinner flasks). Adequate headspace should be provided to facilitate gas exchange (e.g., for a 125 ml shaker flask, use no more than 35 ml culture volume). Shaker flasks should be rotated at 125 - 135 rpm; agitation speed in spinner flasks will depend upon the impeller design. Protect cultures from light.

Adaptation of Cells to Serum-free or Protein-free Media

A sequential adaptation protocol may be necessary if direct adaptation does not work. In both cases, the cells should be in mid-logarithmic growth phase with high (>90%) viability. Success of the adaptation method will depend upon the particular hybridoma cell line and the culture conditions employed. It is recommended that backup cultures in the original medium be maintained until success with the new medium has been achieved.

Direct Adaptation

1. Transfer hybridoma cells growing in serum supplemented medium to serum-free medium, which has been pre-warmed to 37°C. Seeding density should be double the normal seeding density for the cell line. Incubate the cells at 37°C in a humidified atmosphere of 5-8% CO_2.
2. Monitor cell growth until viable cell density reaches 1 x 10^6/ml. Subculture the cells to a viable cell density of 1-2 x 10^5/ml in fresh serum-free medium. Subculture in this manner, monitoring cell growth and viability, for 3 to 5 passages.
3. If the culture fails to maintain acceptable growth and viability over 3-5 passages during direct adaptation, use the sequential adaptation method.

B. Sequential Adaptation

1. Inoculate hybridoma cells at double the normal seeding density in a 75:25 (v/v) mixture of serum supplemented: serum-free medium.
2. Monitor the culture until the density reaches 1 x 10^6 viable cells/ml. Then subculture into a 50:50 (v/v) mixture of serum supplemented : serum-free medium.
3. Monitor the culture until the density reaches 1 x 10^6 viable cells/ml. Then subculture into a 25:75 (v/v) mixture of serum-supplemented : serum-free medium.
4. Monitor the culture until the density reaches 1 x 10^6 viable cells/ml. Then subculture into 100% serum-free medium.
5. It may be necessary to subculture more than once into a given mixture of serum supplemented : serum-free medium until the cells become acclimated. It is advisable to keep a backup culture in the previous media mixture until the cells have adapted.

PromoCell GmbH
Sickingenstr. 63/65
69126 Heidelberg
Germany

North America 1 – 866 – 251 – 2860 (toll free)
Deutschland 0800 – 776 66 23 (gebührenfrei)
France 0800 90 93 32 (ligne verte)
United Kingdom 0800 – 96 03 33 (toll free)
Other Countries +49 6221 – 649 34 0

Email: info@promocell.com
www.promocell.com

Cryopreservation

1. Prepare desired quantity of cells, harvesting in mid-log phase of growth with viability higher 90%.
2. Determine the viable cell density and calculate the required volume of cryopreservation medium (50% fresh medium : 50% conditioned medium + DMSO to a final concentration of 7.5%) to give a final cell density of 0.5 - 1.0 x 10^7 cells/ml. Conditioned medium should be obtained from a high viability, mid-log culture of cells.
3. Prepare the required volume of cryopreservation medium and hold the medium at 4°C until use (make cryopreservation medium on day of intended use).
4. Pellet the cells from culture medium at 100 x g for 5 minutes. Resuspend the pellet in the pre-determined volume of 4°C cryopreservation medium.
5. Dispense aliquots of this suspension into cryovials according to the manufacturer's specifications.
6. Achieve cryopreservation in either an automated or manual controlled rate freezing apparatus following standard procedures (1°C decrease per minute).
7. Frozen cells are stable indefinitely under liquid nitrogen.

Recovery from Cryopreservation

1. Recover cultures from frozen storage by rapid thawing of a vial of cells in a 37 °C water bath with shaking just until the medium thaws.
2. Transfer the entire contents of the vial into the appropriately sized vessel so that the cells are seeded at 5 x 10^5 cells/ml of complete growth medium.
3. Incubate the culture in a humidified atmosphere of 5-8% CO_2 at 37°C +/- 0.5°C
Do not centrifuge the cells as they are extremely fragile upon recovery from cryopreservation.
4. Maintain the culture between 5 x 10^5 and 10 x 10^5 viable cells/ml for the first two subcultures following recovery; thereafter, return to the normal maintenance schedule.

Storage and Stability

Store the Medium at 4 to 8°C in the dark immediately after arrival. Do not freeze. If stored properly, the product is stable until the expiry date stated on the label.

Quality Control

All lots of PromoCell speciality cell culture media are subjected to comprehensive quality control tests. Each lot is routinely tested for growth promotion, absence of cytotoxicity, and physical parameters such as osmolality and pH level. Approved in-house lots of media are used as a reference.
In addition, all lots of media have been tested for the absence of microbial contaminants (fungi, bacteria).

Intended Use

The products are for *in vitro* research use only and not for diagnostic or therapeutic procedures. For safety precautions please see appropriate MSDS.

	HYGM-6 Express C-78410	HYGM-6 Express C-78417 without Phenol Red	HYGM-7 Express C-78440 w/o L-Glutamine	HYGM-7 Express C-78447 without Phenol Red
Unit Size	500 ml	500 ml	500 ml	500 ml
Formulation	Confidential	Confidential	Confidential	Confidential
Specification	Serum-free, with 2,2 g/l $NaHCO_3$, with stable Glutamine	Serum-free, with 2,2 g/l $NaHCO_3$, with stable Glutamine, w/o Phenol Red	Protein-free, with 2,2 g/l $NaHCO_3$, with stable Glutamine	Protein-free, with 2,2 g/l $NaHCO_3$, with stable Glutamine, w/o Phenol Red
Protein content	5 mg/l	5 mg/l	0 mg/l	0 mg/l
Addition required	None, ready-to-use	None, ready-to-use	None, ready-to-use	None, ready-to-use