HB 101®

Catalog Nos. T000, T007+T151

INTENDED USE

HB 101® is a defined serum-free medium designed to support the growth of myelomas, murine and human derived hybridomas, lymphoblastoid cells, particularly the SP2/0 cell line and hybridomas developed from this parental line. HB 101 is ideal for suspension cell growth and subsequent product purification, especially monoclonal antibodies.

PRODUCT DESCRIPTION

When HB 101 is prepared as directed, it provides a complete medium with a protein concentration of approximately 780 ug/mL. All proteins in HB 101 are of human or bovine origin. All human proteins are tested in accordance with FDA approved methodologies and are found non-reactive for HBsAg, HIV and HCV. Bovine proteins are from U.S. source animals BSE has not been found to exist in the U.S.

Cells may be frozen in HB 101 with the addition of a cryopreservative, thawed as needed and inoculated directly back into HB 101. HB 101 is not recommended for cloning unless used in conjuction with feeder cells or a previously screened supplement.

STORAGE AND STABILITY

All HB 101 components should be stored at 2°C to 8°C and used before the expiration date printed on the label of each component. The liguid basal and rehydrated powder media should be protected from fluorescent light.

After reconstitution and sterile filtration, the complete medium should be stored at 2°C to 8°C, protected from fluorescent light and used within three months. Do not freeze the complete medium.

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INSTRUCTIONS FOR USE

Preparing the Liquid Basal Medium from Powder

Transfer the entire contents of the powder basal medium into 9.5 liters (L) of cell culture grade water while slowly stirring. Water produced by distillation, deionization or reverse osmosis purification is suitable for this application.

Dissolve 20 grams of sodium bicarbonate into the solution and adjust the pH to 7.1 ± 0.05 with 1 N HC1 or 1 N NaOH. Add water to a final volume of 10 L and sterile filter into suitable sterile, depyrogenated bottles for storage.

Preparing the Lyophilized Supplement

To reconstitute the lyophilized supplement, add 10 mL of sterile, cell culture grade water and mix by inverting the vial several times. Shaking should be avoided. If necessary, the vial may be placed briefly in a 37°C water bath. The reconstituted supplement may be stored frozen at -10°C or colder for up to 60 days. Avoid repeat freeze-thaw cycles.

Preparing the Complete Medium

The complete medium is prepared by adding the reconstitiuted supplement to the liquid or reconstituted powder basal medium at a ratio of 1:100 (i.e., 1 mL supplement: 99 mL basal medium). L-glutamine should be added to yield a concentration between 1.5 mM and 2.5 mM.

If desired, sodium pyruvate (1.0 mM to 2.0 mM), HEPES (10 mM to 20 mM) and antibiotics may be added at this time.

Transfer of Continous Cell Lines to HB 101® Culture Medium

Cells vary in their ability to transfer from serum-containing to serum-free medium. HB 101 has been demonstrated to allow the direct introduction of a variety of myeloma and hybridoma cell lines from serum-supplemented medium, but adaptation or weaning will be necessary with some cell lines. If, following direct introduction into HB 101, cell growth is low after four passages, a weaning procedure is recommended.

Direct Introduction Method

Harvest cells from serum-supplemented medium. If cells have been frozen in the presence of serum, first initiate culture in serum-supplemented medium until normal growth resumes before transferring them into HB 101. Inoculate cells into HB 101 with a starting density of 4×10^4 to 5×10^4 cells per mL and incubate under normal conditions.

Monitor cells frequently for morphology, viability and growth rate (see Suggestions for Growing Cells in Serum-Free Culture).

Subculture into fresh HB 101 medium when cells reach a density of 2×10^5 to 8×10^5 cells per mL.

Weaning Method

This method allows for a stepwise reduction of serum. Cells should be introduced into HB 101 which has been supplemented with 5% serum. As cell growth permits, reduce the serum concentration to 2.5%, 1% and 0.5%. Finally, place the cells in serum-free HB 101.

Suggestions for Growing Cells in Serum-Free Culture

Cells grown in serum-free conditions are more susceptible to changes in pH and toxic substances in the growth medium. Frequent monitoring is suggested.

For best results, seed HB 101 so that cell concentrations are not less than 4×10^4 cells per mL and subculture before cells reach a density of 1×10^6 cells per mL.

Although HB 101 contains a bicarbonate buffering system, the addition of HEPES or a similar buffer may be beneficial for some applications.