HiDef® PBS/EDTA Cell Dissociation Reagent USER GUIDE

Defined reagent for gentle cell dissociation
Catalog #: LSR-410-125-1, LSR-410-125-10

Product Description

Defined Bioscience's HiDef® PBS/EDTA is a defined reagent for protein-free adherent cell dissociation, for routine aggregate passaging or single-cell suspension. HiDef PBS/EDTA is designed to detach cells from surfaces without impacting cell functionality or surface protein integrity, with applications in stem, tumor, and primary cell culture, among others. HiDef PBS/EDTA can also be used as a cell washing step prior to detachment using other methods, including enzymatic digestion (e.g. trypsinization).

HiDef PBS/EDTA is provided as a 1x solution, 125 mL per bottle, and consists of Dulbecco's Phosphate Buffered Saline (DPBS, without Ca^{2+} and Mg^{2+}) pH 7.4 with 0.5 mM EDTA. Each lot of HiDef PBS/EDTA is used in combination with HiDef® B8 medium in performance testing in a culture assay evaluating using human iPSCs to confirm appropriate and consistent detachment.

Contents and Storage

Content	Catalog #	Amount	Storage	Shelf life
HiDef® PBS/EDTA (1 bottle)	LSR-410-125-1	1 x 125 mL	Store at 2-30°C protected from light	18 months from manufacture date
HiDef® PBS/EDTA (12 bottles)	LSR-410-125-12	12 x 125 mL	Store at 2-30°C protected from light	18 months from manufacture date

Product Usage

Sterility: Use appropriate aseptic technique when handling HiDef® PBS/EDTA. Our HiDef PBS/EDTA is sterile-filtered (0.22 µm PVDF), and so additional sterilization and autoclaving are strongly discouraged. Ensure that all equipment is sterile before use.

Receipt and Preparation: Before using HiDef PBS/EDTA, ensure that the medium is stored at 2-30°C and is within the expiration date. Warm the medium to room temperature or 37°C as needed, depending on your specific application.

Supplementation: HiDef PBS/EDTA is a complete formulation that does not require supplementation. This formulation should be supplemented with other components typically required to support the specific needs of your cells, if any, during use. HiDef PBS/EDTA usage in the context of additional supplements or components will need to be evaluated for your specific cell type and application. The recommended concentration of these other components can vary depending on the cell type and application. Consult the literature or manufacturer's recommendations for the appropriate supplements and concentrations for your application.

pH Adjustment: HiDef PBS/EDTA is prepared to pH 7.4. The pH of this reagent may need to be adjusted depending on any supplements added, the cell type being cultured, and culture/usage conditions. Use a pH meter or pH paper to adjust the pH to the optimal range for your cells using cell culture-grade HCl and/or NaOH as needed.

Cell Culture and Detachment: Follow standard cell culture procedures for seeding, subculturing, and maintaining your cells. Avoid overconfluent cultures or using old medium, as this can lead to cell stress and reduced viability. For standard passaging using HiDef PBS/EDTA, first wash adherent cells with sufficient DPBS, aspirate, and then incubate cells with HiDef PBS/EDTA for 4-5 minutes (for aggregate passaging) or 8-10 minutes or more (for single-cell dissociation). You can monitor colony edges to confirm initiation of detachment. Aspirate HiDef PBS/EDTA, add cell culture medium, use a cell scraper or lifter as needed for full detachment, and recover cells for continued washing and reseeding as needed. See below for general guidance on pluripotent stem cell adherent culture.

For Research Use Only

Storage: HiDef PBS/EDTA can be stored for up to 18 months after the manufacturing date at 2-30°C if protected from light. Once opened, the reagent can be continually reused if appropriate aseptic technique is used. Storage conditions must be adjusted based on manufacturer recommendations if modified with supplemental products.

General Pluripotent Stem Cell Adherent Culture Guide

The following is a guide for passaging pluripotent stem cells (PSCs), including passaging, using HiDef® PBS/EDTA. Modify as needed depending on your cell type and application.

- Use an incubator temperature range of 37 +/- 1°C with humidified atmosphere of 5% CO_2. Ensure that proper gas exchange is achieved in culture vessels. Reduced O_2 tri-gas incubators are encouraged, but not required.
- Split cultures when PSC colonies become too dense, when PSCs show increased differentiation, and/or when colonies cover ~85% of the surface area of the culture vessel, usually every three to five days.
- For standard culture, cells can be passaged at a ratio of up to 1:20 every 4 days after achieving ~70-80% confluence using HiDef PBS/EDTA (Defined Bioscience Catalog # LSR-410-125). The split ratio can vary, though it is generally between 1:2 and 1:4 for newly derived PSCs and between 1:3 and 1:20 for established cultures. Occasionally, cells may recover at a different rate and the split ratio will need to be adjusted.
- A general rule is to observe the last split ratio and adjust the ratio according to the appearance of PSC colonies. If the cells look healthy and the colonies have enough space, split using the same ratio. If the colonies are overly dense and crowded, increase the ratio; if they are sparse, decrease the ratio.
- Newly derived PSC lines may contain a fair amount of differentiation through the first 3-5 passages. It is not necessary to remove differentiated material prior to passaging. By propagating/splitting the cells, the overall culture homogeneity should improve throughout the early passages.
- For complete transition to the HiDef-B8 medium, a minimum two-passage adaptation phase is recommended.

Limited Product Warranty

Defined Bioscience and/or its affiliate(s) warrant their products as set forth in the Defined Bioscience General Terms and Conditions of Sale. If you have questions, please contact Defined Bioscience at info@definedbioscience.com.