INTRODUCTION. The Detachin™ Cell Detachment Solution is a gentle and effective alternative to Trypsin/EDTA for detachment of in vitro cultured cells. It provides a consistent, safe, and efficient method for cell detachment and dissociation. Detachin contains protease and collagenase activities in an isotonic, phosphate buffer solution with EDTA. It has been tested successfully on many different primary cells and cell lines, including: bone marrow cells, stem cells, fibroblasts, hepatocytes, mouse germ cells, keratinocytes, macrophages, chick embryo cells, neuronal cells, vascular endothelial cells, A-375, BHK, CHO, COS, D54, HEK293, HeLa, L-929, M-24, MG-63, MRC-5, NIH-3T3, NT-2 cells, U-251, Vero, SF9 insect cells, and many others. In summary, the Detachin Cell Detachment Solution offers the following benefits:

- Gentle and rapid detachment;
- Maximum cell viability;
- Effective on a wide range of cells;
- No mammalian or bacterial by-products;
- Stable at 4°C for 2 months;
- No need to wash detached cells;
- Convenient and cost effective variety of formats including 100 ml, 10 x 100 ml, and 6-PAC (6 x 50 ml) sizes.

MATERIALS AND METHODS

1. Thaw the Detachin™ Solution at room temperature.
2. After thawing, Detachin can be stored at 4°C for up to two months. For longer-term storage, keep Detachin stored at -20°C.
   NOTE: For steps 3 to 5 below, use aseptic cell culture techniques to avoid contaminating your cells and/or the Detachin Solution bottle.
3. Wash plates, flasks, or beads that contain cells with sterile PBS.
4. Add Detachin to culture dish or flask. Use a minimum of 1.0 ml per 75-cm² surface area and rock container back and forth to cover surface area with Detachin.
   NOTE: Using excess amounts of Detachin will not harm cells or cause any side effects.
5. Incubate culture dish or flask at 37°C and allow cells to detach for 5 to 10 minutes.
6. Count cells, passage, or process as needed.
   NOTE: There is no need to wash the Detachin Solution away or add any enzyme inhibitors to your cells.

LIMITED LICENSE: The purchase price paid for the Detachin™ Cell Detachment Solution (hereto “Detachin”) grants end users a non-transferable, non-exclusive license to use the reagent solution for internal research use only as described in this manual; in particular, research use only excludes and without limitation, resale, repackaging, or use for the making or selling of any commercial product or service without the written approval of Genlantis, a division of Gene Therapy Systems, Inc. (GTS) – separate licenses are available for resale or other commercial applications. The Detachin solution is not to be used for human consumption, diagnostic applications, or included/used in any drug intended for human use.

Care and attention should be exercised in handling the Detachin solution by following appropriate research laboratory practices and kit instructions, including the proper use of protective lab ware during use. MSDS information may be obtained by request to Genlantis at any time.

Purchasers may refuse this license by returning the enclosed materials unused. By keeping or using this kit, you agree to be bound by the terms of this license as governed and enforced by the laws of the State of California.