



## HEPATOCTYE ISOLATION SYSTEM

### *Tissue Dissociation/Cell Isolation*

Most traditional methods published for isolating hepatocytes use crude and partially purified enzyme preparations including various types of collagenase and other proteases. More recently the use of better characterized preparations of collagenase such as Worthington Types 1 and 4 (CLS-1, 4) have provided better results. All crude collagenase preparations can contain lot-variable contaminating proteases, esterases and other enzymes requiring researchers to pre-screen several lots of enzyme and/or continually modify isolation parameters and protocols.

The Worthington Hepatocyte Isolation System has been developed to provide researchers with a reliable, convenient, and consistent hepatocyte cell isolation system. By using the pre-optimized combination of enzymes contained in this kit, it is possible to minimize the lot-to-lot variation and improve the quality of the isolated hepatocytes. In addition, Worthington use-tests each lot by isolating hepatocytes from adult rat to assure performance, reliability, and consistent yield of viable cells.

The method is based on that described by Berry, M.N., modified by Seglen, P.O. (*Methods in Cell Biology, vol XIII*, David M. Prescott ed., Academic Press, 1976; Chapter 4, "Preparation of Isolated Rat Liver Cells", pp 29-83), and further optimized in conjunction with several researchers.

<b>Product</b>	<b>Code</b>	<b>Size</b>	<b>Cat. No.</b>	<b>Price</b>
Hepatocyte Isolation System	HIS	1 Kit	LK002060	\$350.00
<u>Individual Components</u>				
Enzyme Vials	CLSH	1 VI	LK002066	\$43.00
		5 VI	LK002067	193.00
DNase Vials	D2	1 VI	LK003170	\$20.00
		5 VI	LK003172	66.00
10X CMF-Hank's Balanced Salt Solution	HBSS10	500 ML	LK002064	\$60.00
L-15 Media Powder	L15NK	1 x 1L	LK003250	\$22.00
0.15M MOPS Buffer	MOPS	1 x 75 ML	LK002070	\$30.00
7.5% Sodium Bicarb. Solution	NAH	1 x 100 ML	LK002069	\$25.00

### **Description and Package Contents**

The package contains sufficient materials for five separate adult rat liver perfusions or 5-10 adult mouse perfusions. For larger or smaller tissue applications, prepare proportionate volumes of reagents at each step and combine them in the same ratio as described in the protocol.

(Over) [1.11]

**Vial #1:** 10X CMF-HBSS Concentrate, 1 bottle, 500ml Sterile calcium- and magnesium-free Hank's Balanced Salt Solution (CMF-HBSS). The solution is used for washing and perfusing the liver prior to the addition of the dissociating enzyme solution.

**Vial #2: Enzyme Vial** 20,000 Units Collagenase and 30 Units Elastase, 5 Vials Worthington collagenase (Code: CLS-1) and elastase (Code: ESL), filtered through 0.22µm pore size membrane, and lyophilized. Before use, reconstitute with the L-15/MOPS solution and swirl gently to dissolve contents as directed in the following procedure. Store unreconstituted vials at 2–8°C.

**Vial #3: DNase Vial** 1,000 Units DNase I each, 5 Vials Worthington DNase I (Code: D), filtered through 0.22µm pore size membrane, and lyophilized. Before use, reconstitute with L-15/MOPS solution and swirl gently to dissolve contents as directed in the following procedure. Store unreconstituted vials at 2–8°C.

**Vial #4:** 0.15M MOPS, pH 7.5, 1 bottle, 75ml 0.15M MOPS, pH 7.5 buffer concentrate, used to buffer the reconstituted Leibovitz L-15 media.

## References

Alpini *et al.*, "Recent Advances in the Isolation of Liver Cells", *Hepatology* (1994) 20:494-514.

Berry, M.N., Edwards, A.M., and Barritt, G.J.; Isolated Hepatocytes: Preparation, Properties and Applications, RH Burdon and PH Van Knippenberg, eds., Elsevier, Amsterdam, New York, Oxford, Chapt. 2, 1991.

Chen H-L, Wu H-I, Fon C-C, Chen P-J, Lai, M-Y, Chen D-S (1998) Long-term culture of hepatocytes from human adults. *Biomedical Science* 5:435-440

DeRobertis, E.D.P., Saez, F.A. and DeRobertis, E.M.F.: *Cell Biology*, 6th ed., W.B. Saunders Co., Philadelphia, PA, 1975.

Freshney, R. Ian: *Culture of Animal Cells*, Alan R. Liss, Inc., New York, 2000.

Hanks, J.H., and Wallace, R.E.: *Proc. Soc. Exp. Biol. Med.*, 71, 196 1949.

Jakoby, W.B., and Pastan, I.H.: *Methods in Enzymology* Vol. LVIII p. 121, Academic Press, 1979.

**Vial #5:** 7.5% Sodium Bicarbonate (NaHCO<sub>3</sub>), 1 bottle, 100ml 7.5% Sodium bicarbonate concentrate, used to buffer the diluted CMF-HBSS.

**Pouch**, containing Leibovitz L-15 Media Powder, 1 x 1L Reconstitute entire contents of pouch by cutting open top of envelope and pouring contents into beaker containing approximately 800ml of cell culture grade water. Rinse pouch 2 - 3 times with an additional 100ml water. Bring total volume to 1000ml and filter through a 0.22 micron pore size membrane.

## Related Products

**Cell Isolation Optimizing System**  
**Collagenases**  
**Elastase**  
**Hyaluronidase**  
**Neonatal Cardiomyocyte Isolation Kit**  
**Neutral Protease (Dispase®)**  
**Papain**  
**Papain (Neural) Dissociation System**  
**Trypsin**

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Leibovitz, A.: The Growth and Maintenance of Tissue/Cell Cultures in Free Gas Exchange with the Atmosphere, *Am. J. Hyg.*, 78, 173, 1963.

Seglen, P.O., *Methods in Cell Biology*, Vol. XIII, David M. Prescott ed., Ch. 4, pp. 29-83, Academic Press, 1976.

*Worthington Enzyme Manual*, Worthington Biochemical Corp., Freehold, NJ, 1993.

*Worthington Tissue Dissociation Guide*, Worthington Biochemical Corp., Lakewood, NJ, 2003.