Worthington Biochemical Corporation

HEPATOCYTE 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 1-7 have provided better results. All partially purified 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[2], M.N., modified by Seglen[11], P.O., and further optimized in conjunction with several researchers[1, 3-9].

PRODUCT HIGHLIGHT

Description	Code	Catalog No.	Size
Hepatocyte Isolation System	HIS	LK002060	1 Kit
Individual Components			
Enzyme Vials	CLSH	LK002066 LK002067	1 vi 5 vi
DNase Vials	D2	LK003170 LK003172	1 vi 5 vi
10X CMF-Hank's Balanced Salt Solution	HBSS10	LK002064	500 ml
L-15 Media Powder	L15NK	LK003250	1 x 1L
0.15M MOPS Buffer	MOPS	LK002070	1 x 75 ml
7.5% Sodium Bicarb. Solution	NAH	LK002069	1 x 100 ml

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.

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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. 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. 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.

Vial #5: 7.5% Sodium Bicarbonate ($NaHCO_3$),1 bottle, 100ml 7.5% Sodium bicarbonate concentrate, used to buffer the diluted CMF-HBSS.

Pouch, containing Leibovitz[10] 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.

References

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

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