



## PAPAIN DISSOCIATION SYSTEM

### *Tissue Dissociation/Neural Cell Isolation*

Worthington Biochemical Corporation offers a complete kit for the convenient isolation of single, morphologically intact cells for cell culture, flow cytometry or other applications. The method is based upon published techniques using papain to gently dissociate central nervous system (CNS) tissue providing higher yields and better viability than commonly used trypsin procedures. The kit includes five single-use enzyme vials, balanced salt solution, enzyme inhibitor and is functionally tested to assure performance.

<u>Product</u>	<u>Code</u>	<u>Size</u>	<u>Cat. No.</u>	<u>Price</u>
Papain Dissociation System	PDS	1 Kit	LK003150	\$ 175.00
		3 Kits	LK003153	425.00
Papain Dissociation System (wi buffer/EBSS)	PDS2	1 Kit	LK003160	\$ 150.00
		3 Kits	LK003163	390.00
<u>Individual Components</u>				
Papain Vials	PAP2	1 VI	LK003176	\$ 18.00
		5 VI	LK003178	63.00
DNase Vials	D2	1 VI	LK003170	\$ 17.00
		5 VI	LK003172	62.00
Inhibitor-BSA Vial	OI.BSA	1 VI	LK003182	\$ 55.00
Earle's Buffer Vial	EBSS	1 VI	LK003188	\$ 45.00

Proteolytic enzymes are widely used in cell dissociation.<sup>1</sup> With some tissues papain has proved less damaging and more effective than other proteases. Lam<sup>2</sup> found that of the enzymes used for dissociating turtle retina, papain produced the least trauma. Intact single photoreceptor cells have been isolated from adult salamander retina with papain.<sup>3,4</sup> Huettner and Baughman described a method using papain to obtain high yields of viable, morphologically intact cortical neurons from postnatal rats.<sup>5</sup> Finkbeiner and Stevens<sup>6</sup> applied the Huettner and Baughman method to the dissociation of postnatal rat hippocampus. Papain is used with fetal as well as postnatal brain regions to provide maximal dissociation and viability of neurons.<sup>7</sup>

#### Description of Package and Contents

The Worthington Papain Dissociation System is a set of reagents intended for use in the tissue dissociation method of Huettner and Baughman. The materials are designed for convenience and simplicity and are useful to the occasional user as well as the more experienced and frequent user. Each lot is use tested for performance in neural tissue dissociation and provides five single use vials in order to have freshly prepared enzyme solutions for each dissociation.

(Over)

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The package contains sufficient materials for dissociation of five separate tissue aliquots of up to 0.3 - 0.4 cm<sup>3</sup> each. For larger tissue samples prepare proportionately larger volumes of reagents at each step and combine them in the same ratio as described in the protocol. Individual component vials are also available for modified preparations in addition to a complete kit without EBSS for specialized buffer requirements.

#### Vial 1

Sterile Earle's Balanced Salt Solution (EBSS) with calcium, magnesium, bicarbonate and phenol red, 1 x 100ml per package.

Aliquots of this vial are used to reconstitute other vials and to prepare dilute inhibitor solution. Refrigerate between uses and equilibrate with sterile O<sub>2</sub>:CO<sub>2</sub> before each use.

#### Vial 2

Papain containing L-cysteine and EDTA, five single use 100 unit vials per package.

This material is 0.22µm membrane filtered and lyophilized in autoclaved vials. A vial reconstituted with five mls of EBSS (vial 1) yields a solution at 20 units of papain per ml in one millimolar L-cysteine with 0.5mm EDTA. Brief incubation is needed to insure full solubility and activity.

#### Vial 3

Deoxyribonuclease I (DNase), five single use 1,000 unit vials per package.

This material is 0.22µm membrane filtered and lyophilized in autoclaved vials. A vial reconstituted with 0.5ml of EBSS (vial 1) yields a solution at 2000 units of deoxyribonuclease per ml. Avoid vigorous mixing.

#### Vial 4

Ovomucoid protease inhibitor with bovine serum albumin, one vial per package.

This material is 0.22µm membrane filtered and lyophilized in autoclaved vials. A vial reconstituted with 32ml of EBSS (vial 1) yields a solution at an effective concentration of 10mg of ovomucoid inhibitor and 10mg of albumin per ml. The inner rubber stopper can be discarded after reconstitution. Aliquots of this vial are used for each dissociation. Refrigerate between uses and equilibrate with sterile O<sub>2</sub>:CO<sub>2</sub> before each use. Stable after reconstitution when stored at 2-8°C.

Also included is a card correlating color with pH for use as a guide in O<sub>2</sub>:CO<sub>2</sub> equilibration.

#### **Procedure Overview**

Briefly the procedure is as follows: Components of the dissociation medium are reconstituted as described in the package insert; minced tissue is added and the mixture is equilibrated with O<sub>2</sub>:CO<sub>2</sub>. Tissue is dissociated by incubation

with activated papain at 37°C, followed by trituration. Dissociated cells are pelleted then resuspended in medium containing ovomucoid, a protease inhibitor. Intact cells are separated from cell membranes by centrifugation through a single step discontinuous density gradient and the pellet finally resuspended in medium appropriate for cell culture or flow cytometric analysis.

#### **References:**

1. Bashor, Mark M.: *Methods in Enzymology, Vol LVIII* (Colowick, S.P., and Kaplan, N.O., eds.) pg 124, Academic Press, New York (1979).
2. Lam, Dominic M. K.: Biosynthesis of Acetylcholine in Turtle Photoreceptors, *Proc. Nat. Acad. Sci. (USA)*, 69, 1987 (1972).
3. Bader, C.R., MacLeish, P.R., and Schwartz, E.A.: Responses to Light of Solitary Rod Receptors Isolated from Tiger Salamander Retina, *Proc. Nat. Acad. Sci. (USA)*, 75, 3507 (1978).
4. Townes-Anderson, E., MacLeish, P.R., and Raviola, E.: Rod Cells Dissociated from Mature Salamander Retina: Ultrastructure and Uptake of Horseradish Peroxidase, *J. Cell Biology*, 100, 175 (1985).
5. Huettner, James E., and Baughman, Robert W.: Primary Culture of Identified Neurons from the Visual Cortex of Postnatal Rat, *J. Neuroscience*, 6, 3044 (1986).
6. Finkbeiner, Steven, and Stevens, Charles F.: Applications of Quantitative Measurements for Assessing Glutamate Neurotoxicity, *Proc. Nat. Acad. Sci. (USA)*, 85, 4071 (1988).
7. Dreyfus, Cheryl F., and Black, Ira B.: *Methods in Neuroscience, Vol 2* pg 10, P. Michael Conn, ed., Academic Press, Inc., San Diego (1990).

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