Product Information

Collagenase-DNase I-Dispase II Blend

B20223

Product Description

The tissue dissociation process consists in the detachment of the extracellular matrix of animal tissue and the isolation of viable and functional cell, with minimal impact, for tissue culture use.[1,2,3,5]

Collagenase-DNase I- Dispase II blend is a tissue dissociation enzyme blend, combined with Collagenase from Clostridium histolyticum, Deoxyribonuclease I from Bovine Pancreas and Dispase II from *Bacillus Polymyxa*.

The main enzyme used for tissue dissociation is Collagenase. Collagenases (Clostridiopeptidase A) are metalloproteinases involved in the degradation of the extracellular matrices of animal cells, due to their ability to digest native collagen under physiological conditions that holds animal tissues together.[6,7] Collagenase from Clostridium histolyticum is mainly used for the dissociation of tissues for the establishment of primary cell cultures.[8]

The second enzyme found in the blend is Deoxyribonuclease I (DNase I- Deoxyribonucleate 5'-oligonucleotido-hydrolase).

DNase I is a double-strand specific endonuclease that degrades DNA. Bovine pancreatic deoxyribonuclease I (DNase I) is a DNA minor grove-interacting nuclease, which shows relatively low specificity. During tissue dissociation, parts of the cells are lysed resulting in a release of DNA. Monomolecular DNA may cause clumping of cells.[10] Addition of DNase I to the dissociation buffer leads to a degradation of this extracellular DNA, thereby avoiding the loss of cells from undesired clumping.[11,12]

The third enzyme in the cocktail is Dispase II. Dispase is a rapid, effective, gentle, and neutral protease. The enzyme preserves the viability of the epithelial cells while cleaving the basement membrane region. It can also be used to prevent clumping in suspension cultures. This protease cleaves fibronectin and type IV collagen, but not laminin, type V collagen, serum albumin, or transferrin.[13]

Collagenase-DNase I- Dispase II blend is an important tool in tissue dissociation research field. It can be used for the effective dissociation of tissues and the isolation of single-cells preparations required in assays

Reagent

This product is supplied as a lyophilized powder.

Preparation Instructions

To receive the enzymes activity, describe in the Certificate of Acceptance (COA):

> Reconstitute the content of a vial with cold 10 ml of Hanks' Balanced Salt solution (HBSS) modified, without calcium chloride and magnesium sulfate (H6648). Mix the vial by inversion until all the lyophilized product is diluted in Hanks' Balanced Salt solution.

After reconstitution, the solution will contain approximately 1 mg/mL Collagenase and 0.1 mg/mL DNase I.

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

Store the lyophilized product at –20 °C. The product retains its activity for 2 years in the supplied form. It is not recommended repeated freezing and thawing since activity decreases after reconstitution

Product Profile

Enzymatic activity of 10 mL/vial reconstituted solution equivalent to:

Collagenase enzymatic activity: 1-5 units/mL

Deoxyribonuclease I enzymatic activity: 2000-20000 Kunitz units/mg

Dispase II enzymatic activity: 2-10 units/mL

Unit Definition

DNase enzymatic activity

One Kunitz unit will produce a ΔA260 of 0.001 per minute per mL at pH 5.0 at 25 °C, using DNA (D3664) as substrate, with [Mg2+] = 4.2 mM.[4]

Collagenase enzymatic activity

One unit hydrolyzes 1.0 micromole of FALGPA (Furylacryloyl-Leu-Gly-Pro-Ala, F5135) per minute at pH 7.5 at 25 °C in the presence of calcium ions.

Dispase II enzymatic activity

One unit will hydrolyze casein to produce color equivalents to 1.0 umole of Tyrosine per minute at pH 7.5 at 37 °C (color by Folin-Ciocalteu reagent)

Note: To obtain the best results in different techniques and preparations we recommend on determining optimal working concentration by calibration test.

References:

1. Grabert, K. et al., Macrophages 77–86 (2018)
2. Lee JK, Tansey MG. Methods Mol Biol. , 1041:17-23. (2013)
3. Fujiyama S. et al., Int Immunol. 6; 31(1):51-56 (2019)
4. Kunitz, M., J. Gen. Physiol., 33(4), 349-362 (1950).
5. Holmbeck, K.; Birkedal-Hansen, H.
6. Encyclopedia of Biological Chemistry, 542-544 (2013)
7. Harold E. Van Wart, in Handbook of Proteolytic Enzymes (Third Edition),607-611 (2013)
8. Brondon Y.H.Chan et al, Encyclopedia of Signaling Molecules, 5794-5794 (2018)
9. Fazzina R et al, Transfusion, 55 (12): 2864-73 (2015)
10. MP Curry, J. of immunological methods, Vol 242 (1-2): 21-31 (2000)
11. Reichard A, Cytometry 95(2): 219-226 (2019)
12. Singer BD, American journal of physiology. Lung cellular and molecular physiology, 310 (9): L796-801(2016)
13. Ning Li, Front Immunol. 4 (61), 2013
14. Stenn K S, J Invest dermatol, 93(2): 287-290 (1989)

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