

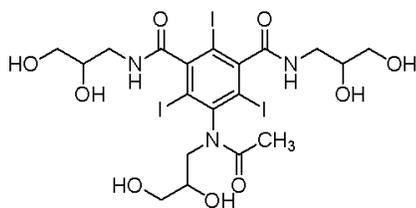
## Product Information

### HistoDenz™

Catalog Number **D2158**  
Store at Room Temperature

CAS RN 66108-95-0

Synonym: 5-(N-2,3-Dihydroxypropylacetamido)-2,4,6-triiodo-N,N'-bis(2,3-dihydroxypropyl)isophthalamide



### Product Description

Molecular Formula: C<sub>19</sub>H<sub>26</sub>I<sub>3</sub>N<sub>3</sub>O<sub>9</sub>

Molecular Weight: 821.14

$\lambda_{\text{max}}$ : 244 nm,  $E^{\text{mM}} = 342$  (H<sub>2</sub>O)

HistoDenz™ is a non-ionic X-ray contrast compound developed specifically as a density gradient medium. This compound shares a number of characteristics with a related compound metrizamide. However, the stabler, more inert nature of HistoDenz endows it with a number of favorable properties as a gradient medium. It has very low toxicity, low osmolality, high density, and low viscosity (compared to sucrose). It is suitable for separating everything from macromolecules to viable cells by either rate-zonal or isopycnic centrifugation.<sup>1</sup>

Non-aqueous denaturing gradients up to 50% (w/v) with a maximum density of 1.4 g/ml for rate zonal and isopycnic separations can be prepared.<sup>2</sup> HistoDenz may be removed from samples by recentrifugation in appropriate buffer, dialysis, ultrafiltration, gel filtration, or sample precipitation with TCA or ethanol.<sup>2</sup>

### 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.

### Preparation Instructions

This product is soluble in water (50 mg/ml), yielding a clear, colorless solution. See Table 1 for properties of HistoDenz solutions.

It is also soluble in formamide and dimethylformamide. Non-aqueous denaturing gradients up to 50% (w/v) with a maximum density of 1.4 g/ml can be prepared for rate zonal or isopycnic separations.<sup>2</sup>

### Storage/Stability

Store the product at room temperature.

Solutions kept sterile and protected from light are suitable for use for 5 years. Solutions are heat stable and can be autoclaved or sterile filtered. The stability to autoclaving is enhanced by the addition of millimolar concentrations of Tris and Na<sub>2</sub>CaEDTA.<sup>1</sup>

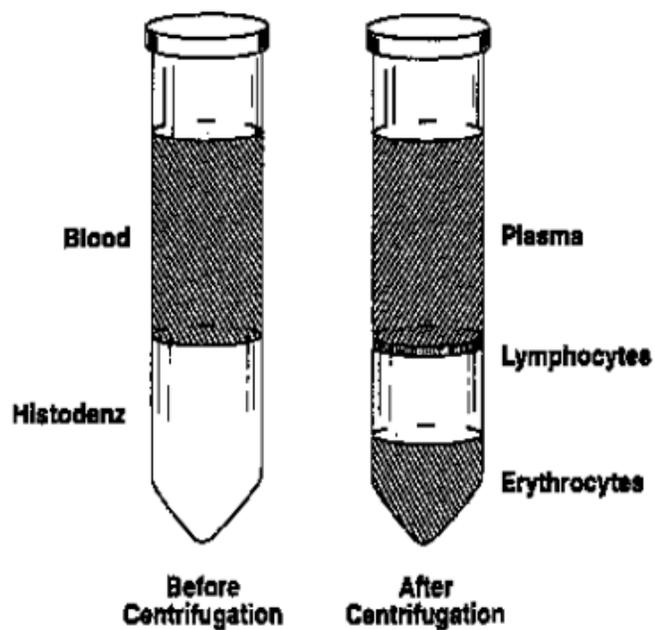
### Procedure

For isolation of human lymphocytes, prepare a 14.1% (w/v) HistoDenz Solution containing 0.44% (w/v) NaCl and 5 mM tricine, pH 7.2. The entire procedure should be performed at room temperature.

1. Mix an equal volume of anticoagulated blood with physiological saline (0.9%).
2. In a 15 ml centrifuge tube, carefully layer 6 ml of the diluted blood from step 1 over 3 ml of the 14.1% HistoDenz Solution. Avoid mixing of the two layers. Cap the tube (see Figure 1).
3. Centrifuge at 800 × g for 25 minutes at room temperature in a swingout rotor.

**Note:** Lymphocytes will form a white layer at the interface region between the plasma and the HistoDenz Solution (see Figure 2).

4. Remove the supernatant to a level 3–4 mm above the white band. Then carefully collect the lymphocytes with a plastic pipette. To ensure complete removal of lymphocytes, move the pipette over the whole cross sectional area of the layer interface and collect the HistoDenz layer to a level ~2 mm above the erythrocyte layer.
5. Lymphocytes may be concentrated by diluting with physiological saline and centrifuging at 400 × g for 10 minutes.



**Figure 1**

**Figure 2**

**References**

1. Steensgard, J., et al., Preparative Centrifugation, ed. Rickwood, D., IRL Press Ltd. (New York, NY: 1996) pp. 9, 91.
2. Rickwood, D. et al., Nycodenz: a new nonionic iodinated gradient medium. *Anal. Biochem.*, **123(1)**, 23-31 (1982).

HistoDenz is a trademark of Sigma-Aldrich®  
Biotechnology LP and Sigma-Aldrich Co.

ALF,NSB,MAM 03/08-1

**Table 1.**  
Solution Properties of Histodenz<sup>2</sup>

Percent (w/v)	Molarity	Refractive Index (20 °C)	Density (g/ml, 20 °C)	Osmolality (mOsm)	Viscosity (cP at 20 °C)
0	0	1.3330	0.999	0	1.0
10	0.122	1.3494	1.052	112	1.3
20	0.244	1.3659	1.105	211	1.5
30	0.365	1.3824	1.159	299	1.8
40	0.487	1.3988	1.212	388	3.2
50	0.609	1.4153	1.265	485	5.3
60	0.731	1.4318	1.319	595	9.5
70	0.853	1.4482	1.372	1045	17.2
80	0.97	1.4647	1.426	—	30.0

Sigma brand products are sold through Sigma-Aldrich, Inc.

Sigma-Aldrich, Inc. warrants that its products conform to the information contained in this and other Sigma-Aldrich publications. Purchaser must determine the suitability of the product(s) for their particular use. Additional terms and conditions may apply. Please see reverse side of the invoice or packing slip.