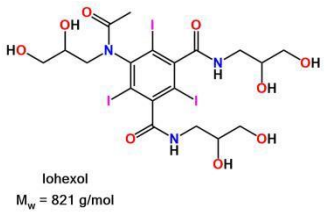
Nycodenz®

INTRODUCTION



Nycodenz® is a non-ionic tri-iodinated derivative of benzoic acid with three aliphatic hydrophilic side chains. Nycodenz® is the trademark name for iohexol, whose systematic name is 5- (N-2, 3-dihydroxypropylacetamido)-2, 4, 6-tri-iodo-N, N'-bis (2, 3 dihydroxypropyl) isophthalamide.

Technical data of analytical grade	
Min. content Nycodenz®	Min. 98 %
Max. O.D. 420 nm	0.07
H_2O (Karl Fischer titration)	Max. 5 %
Inorganic iodide	Max. 20 µg/g

CHARACTERISTICS

Nycodenz® has a molecular weight of 821 g/mol and a density of 2.1 g/ml. Nycodenz® is non-ionic, non-toxic and is very water soluble. Stock solutions of over 80 % (w/v) can be prepared. Nycodenz® is also soluble in formamide and dimethyl formamide, thus it is possible to prepare non-aqueous denaturing gradients of Nycodenz®. Aqueous solutions of Nycodenz® have a very high water activity. Most particles will therefore be fully hydrated in solutions of Nycodenz® and will band at a low density. Solutions of Nycodenz® are stable to heat and may be autoclaved, stability to autoclaving is enhanced by the addition of small amounts of Tris and EDTA.
Solutions of Nycodenz® are very resistant to bacterial degradation and Nycodenz® is not metabolized by mammalian cells.
The concentration and density of solutions of Nycodenz® can easily be determined by measuring the refractive index. The relationship between concentration, refractive index (n) and density is linear and can be formulated:
Concentration, % (w/v) = 607.75 n - 810.13
Density (g/ml) = 3.242 n - 3.323
Before using this equation, the refractive index must be corrected for the presence of buffer or salt in the gradient medium.
Nycodenz® is a non-particulate medium; therefore the distribution of cells in a gradient can be determined using a haemocytometer, electronic particle counter or by light-scattering measurements using a spectrophotometer.

STABILITY AND STORAGE

Nycodenz® in solid form is stable for a period of 5 years when stored at room temperature and protected from light. Nycodenz® in solution is stable for 5 years provided that it is kept sterile and protected from light. Prolonged exposure to direct sunlight leads to release of iodine from the molecule. This effect is negligible when working with these solutions on a day to day basis.

APPLICATIONS

Nycodenz® can be used in the fractionation of nucleic acids, proteins, polysaccharides and nucleoproteins. Moreover, most types of subcellular organelles can be successfully isolated on gradients of Nycodenz® under either isotonic or mildly hypertonic conditions. Nycodenz® has a low osmolality, and is non-toxic, thus making it an ideal medium for the separation of intact living cells. The low viscosity of Nycodenz® in parallel with it's non-ionic characteristics has proved Nycodenz® to be useful in the isolation and purification of viruses and bacteria.

FORMATION OF GRADIENTS

Gradients of Nycodenz® can be generated in the following ways:

1. Formed in situ by centrifugation (self-forming gradients).
2. Layering solutions of the desired concentration into an appropriate centrifuge tube and allowing the solutions to diffuse. Using Nycodenz® isotonic solution gradients can be simply prepared within 45 minutes (see next section).
3. Freezing and thawing.
4. Gradient mixers.

PREPARATION OF ISOTONIC GRADIENTS OF NYCODENZ®

Gradient solutions for the preparation of essentially iso-osmotic Nycodenz® gradients has been devised and these can be prepared using an iso-osmotic solution of Nycodenz® which contains 27.6% (w/v) Nycodenz® (density = 1.15 g/ml) made up in buffered medium. This solution may be diluted to desired concentration by using a buffered diluent containing either sucrose or NaCl as osmotic balancer.
The composition of these diluents are as follows:
0.75 g NaCl or 7.45 g sucrose dissolved in 100 ml 5 mmol/l Tris-HCl (pH 7.5) containing 3 mmol/l KCl and 0.3 mmol/l $CaNa_2EDTA$.
The relationship between density and refractive index (n) can

NaCl diluent	**Sucrose diluent**
Density = 3.287 n - 3.383	Density = 3.410 n - 3.555

COMPATABILITY WITH SOME WIDELY USED ASSAYS

Nycodenz® does not interfere with the orcinol and diphenylamine reactions for estimation of nucleic acids, nor with the very sensitive dyebinding assays for protein and DNA. Polysaccharides and sugars can be determined in the presence of Nycodenz® using the phenol/H_2SO_4 assay. Fluorimetric assays of nucleic acid and proteins can also be carried out in the presence of Nycodenz®.
Nycodenz® does not interfere with most assays for the marker enzymes of subcellular components, also most commercial scintillants are compatible with Nycodenz®.

REMOVAL OF NYCODENZ® FROM SAMPLES

Nycodenz® can be removed from samples by dialysis, ultrafiltration and gel filtration. Cells, subcellular organelles and other particulate matter can be separated from Nycodenz® by centrifugation without the risk of contaminating the pellet with Nycodenz®.
Nycodenz® is readily soluble in both acidic and ethanolic media. Thus, in a number of cases samples can be isolated free of Nycodenz® by precipitating the sample with trichloroacetic acid or ethanol.

ORDERING INFORMATION

Nycodenz®	prod. no. 18003	1 x 500 g

For Research Use Only.

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