AXIS-SHIELD

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BIOLOGICAL SEPARATIONS

NEWS BULLETIN FOR AXIS-SHIELD DENSITY GRADIENT MEDIA

The risks and problems of using Percoll® for the isolation of cells – comments from publications

Important properties of gradient media

The standard solution of Percoll® that is used for most cell separations is a colloidal silica suspension that is stabilized by polyvinylpyrrolidone (PVP). The solution contains trace amounts of free PVP, which is a known toxin. Percoll® is not endotoxin-tested, but measurements indicate that the levels are at least 6 EU/ml.

In contrast all Axis-Shield density gradient media are produced in facilities under strict FDA and EU cGMP compliance to the EU Pharmacological Standard of <1 EU/ml. Certificates of Analysis are available for every batch and the measured endotoxin levels are routinely <0.13 EU/ml.

The importance of low endotoxin levels is indicated by the following effects of lipopolysaccharide: *In vivo* endotoxin (lipopolysacchsaride) causes a variety of inflammatory responses, hypotension, changes to leukocyte populations, intravascular coagulation, shock and death. *In vitro* endotoxin interacts with CD14 and other receptors; causes cytokine production in monocytes and macrophages; activates complement and coagulation cascades and acts as a B cell mitogen.

The wide range of mammalian and non-mammalian cells that demonstrate sensitivity to Percoll® suggests that possible effects on any cell type from this medium may need to be considered. The text below describes some of the specific problems (purity, yield and/or function) that have been encountered with Percoll® gradients.

General effects on mammalian cells

One of the first publications which warned about the possible deleterious effects of Percoll® was published over 25 years ago by Wakefield J.S. et al [1] who observed that peritoneal macrophages, hepatic Kupffer cells and testis Leydig cells phagocytosed the colloidal silica particles. Even though phagocytosis can be reduced at 4°C, the adherence of macrophages to plastic was inhibited by the Percoll® at this temperature and the authors suggested that a number of surface phenomena may be affected by a loose association of the colloidal silica particles to the cell surface.

Lung cells

Viscardi, R.M. et al [2] were concerned that Percoll®, a gradient medium that is commonly used for type II pneumocyte cell isolation, may be toxic to cells. They favoured the use of Nycodenz®, emphasizing that it is a true solution, transparent, not metabolized by cells, and nontoxic to cells.

Gastric parietal cells

Benn et al [3] used continuous gradients favoured the use of Nycodenz® for these cells. Purities >95% were obtained; Percoll® gave much inferior recoveries and purity of parietal cells and also stimulated acid and cAMP secretion by gastric cells. More recently the method has been OptiPrep™, which gave even greater purity [4].

Progenitor cells

Mayanagi et al [5] emphasized the lack of toxicity of Nycodenz®, compared to Percoll®, and the avoidance of positive selection with antibody-beads was important in recovering viable cells.

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Erythrocytes

The deformability and rigidity of reticulocytes and erythrocytes may be compromised by the use of Percoll®. There is evidence that the colloidal silica particles adhere to the surface of erythrocytes (even after washing) and cause progressive hemolysis [6].

Bacteria

The recovery, purity and functional integrity of the bacteria isolated from soil in Nycodenz® is superior to isolation in CsCl and Na_2WO_4 and Percoll® [12,13]. Isolation of obligate intracellular bacterium in OptiPrep™ has shown better yields and improved resolution from host cell components than with Percoll® [14].

Sea urchins

For the fractionation of coelomocytes sucrose gradients pose the problem of high osmolality; the silica particles of Percoll® may be engulfed by potentially phagocytic cells and they also tend to adhere to cell surfaces. The cells also have an extreme sensitivity to lipopolysaccharide [15.] OptiPrep™ posed none of these problems and provided excellent resolution of the different cell types.

Protozoa

Nycodenz® gradients were shown to be superior to those of Percoll® for *Cryptosporidium* oocyst purification, when considering both recovery and particulate load [7]. Iodixanol gradients demonstrate an ability to resolve different cell types that is lacking in other density gradient media in the case of both *Sarcocystis neurona* [8] and *Enterocytozoon bieneusi* [9]. The use of Nycodenz® to enrich for either gametocytes or ookinetes from cultures of *Plasmodium falciparum* greatly improved the viability compared to those purified in Percoll® [10]. Although Percoll® gradients were able to provide a purified sporocyst fraction from *Toxoplasma*, because these particles do not all band in a discrete manner in such gradients, they were unable to provide the simultaneous isolation of a pure oocyst wall fraction possible with OptiPrep™ [11].

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