BIOTECH SUPPORT GROUP

NuGel™

Polymer Coated Silica Affinity Matrices

- Non-specific sites are virtually eliminated by a polymer coating
- Stable across a wide pH range 2 – 10
- 1000Å, 50µm Silica suitable for LC and batch processes
- Applications include: Enzyme immobilization, separation and purification of biopolymers, immobilization of proteins & ligands – monoclonal antibodies, hormones, peptides, haptens, drugs, etc

Silica has been an industry standard as an advantageous matrix suitable for high performance liquid chromatography. With NuGel™, non-specific sites have been virtually eliminated making it an ideal support for affinity purification. Through a proprietary polymer coating, Silica is cross linked forming a reactive Poly-Epoxy functionality stable across a wide pH range (pH 2 to 10). From this foundational chemistry, all of the NuGel™ affinity products are derived.

For Immobilization of Proteins, Antibodies, Hormones, Peptides, Haptens, Drugs, Etc.						
Product Name	**Matrix Reactive Group**	**Ligand Reactive Group**	**Special Features**	**Size**	**Column Volume Approx-imately**	**Item No.**
NuGel™ Poly-Epoxy	Terminal Epoxy	Amino	Direct Coupling of Amino Groups	25 Grams	50 ml	NPEY-25
NuGel™ Poly-Amine	Terminal Amine	Carboxylic Acid, or Carbohydrate	Carbodiiamide reaction, or $NaIO_4$ derived Aldehyde	25 Grams	50 ml	NPAM-25
NuGel™ Poly-Aldehyde	Terminal Aldehyde	Amino	Direct Coupling of Amino Groups	25 Grams	50 ml	NPAY-25
NuGel™ Poly-Hydroxy	Terminal Glycol	Amino	Carbodiimidazole mediated reaction	25 Grams	50 ml	NPHX-25
NuGel™ Poly-Carboxy	Terminal Carboxylic Acid	Amino	Carbodiiamide mediated reaction	25 Grams	50 ml	NPCY-25
NuGel™ Poly-NHS	Terminal N-Hydroxy Succinimide	Amino	Direct Coupling of Amino Groups	25 Grams	50 ml	NPHS-25

* Kilogram quantities and other particle sizes and porosity of NuGel™ are also available upon request.

NuGel™ Poly-NHS Protocol

NuGel™ Poly-NHS is a derivative of NuGel™ polyamino affinity support. This affinity support contains NHS groups at the end of hydrophilic spacer arms and is used to couple ligands containing amino groups.

Technical Data	
Spacer Arm	Polymerized hydrophilic carbon chain
Porosity	1000Å
Average Particle Size	50um

Substitution Level	100-200 uEq/gm of NHS groups

Special Features:

- Couples ligands containing free amino groups.
- pH stable from 2 to 9.

Poly-NHS Protocol for Aqueous Immobilization

(contact Technical Services for Anhydrous Immobilization)

1. N-Hydroxy Succinimide (NHS) derivatives readily react with amine nucleophiles to yield covalently coupled proteins in aqueous solutions. The unreacted groups undergo spontaneous hydrolysis to their carboxyl derivatives. For immobilizing proteins, optimal coupling takes place using high protein concentrations, 10-20 mg/ml, but good results can be achieved with 1-2 mg/ml. Typically protein coupling ranges from 5 to 10 mg/ ml column volume. Suitable coupling buffers are:
   0.1 M MOPS, pH 7,
   0.1-0.2 M Phosphate, pH 7.5,
   0.1-0.2 M $NaHCO_3$, pH 8, prepared fresh,

   Do not use Tris or Glycine buffers as they contain amines.

2. One gram of NuGel™ produces approximately 2 ml column (or bed) volumes of gel. Based on 1 gram of NuGel™, add 10 ml of ice-cold DI water or coupling buffer. Filter quickly on Buchner funnel. Transfer wet cake to protein solution.

3. Mix at 4°C overnight using orbital shaker. Do not use magnetic stirrer.

4. Wash with 5-10 volumes of fresh cold coupling buffer. Store in coupling buffer at 4°C in a well-sealed container.

Note: After coupling, to measure the unreacted protein by UV, the solution must be dialyzed.

Operating Modes

Since the support matrix is based on a rigid 50 µm particle, NuGel™ can be operated in low pressure pump or gravity flow columns, or in batch mode.

NuGel™ Related References

Patents

1. Monoclonal antibodies directed to the cytotoxic lymphocyte maturation factor European Patent EP0790255
2. Purification of Immunoglobulins Using Affinity Chromatography and Peptide Ligand United States Patent Application 20090005261
3. Purification and characterization of cytotoxic lymphocyte maturation factor and monoclonal antibodies thereto United States Patent Application 20030204059
4. Cytotoxic lymphocyte maturation factor European Patent EP0433827
5. Methods for nucleic acid isolation and kits using a microfluidic device and concentration step United States Patent 7939249RV
6. Purification of immunoglobulins using affinity chromatography and peptide US 2006/0153834 A1
7. Purification of immunoglobulins using affinity chromatography and peptide ligands United States Patent 20090005261
8. Maturation Factor (CLMF) which is produced and synthesized by human NC-37 B lymphoblastoid cells United States Patent 2003/0204059

Affinity

1. Dermot Walls, Robert McGrath and Sinéad T.Loughran A Digest of Protein Purification. *Methods Molecular Biology.* Volume 681: 3-23 (2011)
2. Ehrlich, G. K., Michel, H., Chokshi, H. P. and Malick, A. W. Affinity purification and characterization of an anti-PEG IgM. *Journal of Molecular Recognition*, 22: 99-103 (2009).
3. Development of hepatitis B virus capsids into a whole-chain protein antigen display platform: New particulate Lyme disease vaccines. *International Journal of Medical Microbiology* Volume 298, Issues 1-2, 3 January 2008, Pages 135-142
4. A sensitive and high-throughput assay to detect low-abundance proteins in serum Hongtao Zhang, Xin Cheng, Mark Richter & Mark I Greene. *Nature Medicine* 12, 473 - 477 (2006)
5. Transformation of a L-peptide epitope into a D-peptide analog. *Peptides Frontiers of Peptide Science American Peptide Symposia*, 2002, Volume 5, Session XI, 769-770
6. Expression and folding of an antibody fragment selected in vivo for high expression levels in Escherichia coli cytoplasm. *Research in Microbiology* Volume 153, Issue 7, September 2002, Pages 469-474
7. Identification of model peptides as affinity ligands for the purification of humanized monoclonal antibodies by means of phage display *Journal of Biochemical and Biophysical Methods* Volume 49, Issues 1-3.2001
8. George K. Ehrlich, Pascal Bailon, Wolfgang Berthold. Phage Display Technology - Identification of Peptides as Model Ligands for Affinity Chromatography *Affinity Chromatography Methods in Molecular Biology*, 2000, Volume 147, 209-220
9. A Digest of Protein Purification and partial amino acid sequence of a 28 kDa cyclophilin-like component of the rat liver sigma receptor *Life Sciences* , Volume 55, Issue 8, 1994.
10. Nachman, M., Azad, A. R. M. and Bailon, P. (1992), Efficient recovery of recombinant proteins using membrane-based immunoaffinity chromatography (MIC). *Biotechnology and Bioengineering*, 40: 564-571.
11. Kinetic aspects of membrane-based immunoaffinity chromatography Journal of Chromatography A Volume 597, Issues 1-2, 24 April 1992, Pages 167-172
12. Identification of model peptides as affinity ligands for the purification of humanized monoclonal antibodies by means of phage display. Methods in Molecular Biology, 2000, Volume 147, 209-220
13. Membrane-based receptor affinity chromatography Journal of Chromatography A Volume 597, Issues 1-2, 24 April 1992, Pages 155-166 9th International Symposium on Affinity Chromatography and Biological Recognition

Ion Exchange

1. Levin W Protein Purification of recombinant human secretory phospholipase A2 (group II) produced in long-term immobilized cell culture. *Expr Purif* 1992 Feb;3(1):27-35.

Contact Us

We welcome your questions and comments regarding our products.

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