Applications
Update... PolyAspartic Acid WCX (PolyCAT A™)

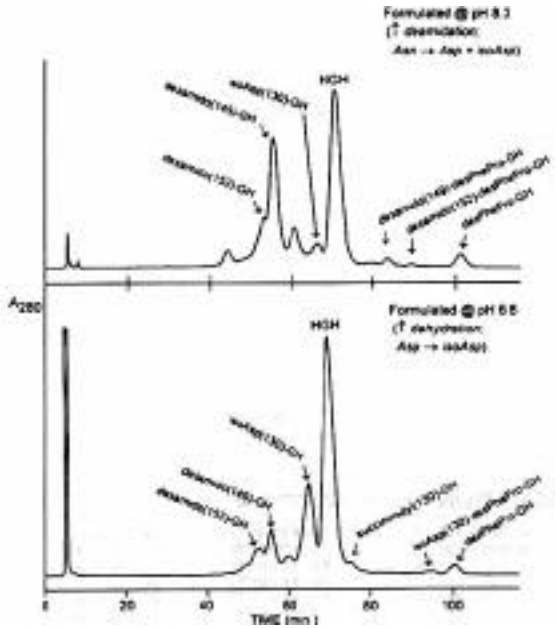
Protein Variant Analysis:
Selective Separations for Quality Control and R&D

Advances with the PolyCAT A™ columns make it possible to separate and quantitate many protein variants that differ by a single residue. In the past, such detection usually required protein digestion and peptide mapping. Variants form as purified recombinant proteins degrade in storage, the rate and pathway is influenced by the solution pH. At high pH, the main pathway is ***deamidation*** of susceptible Asn- residues to yield a succinimide ring. This hydrolyzes to a racemic mixture of Asp- and ***iso*Asp-** residues. At neutral pH, the fastest pathway is ***dehydration*** of Asp- to yield first a succinimidyl- intermediate, then Asp- and isoAsp-. The effects on biological activity range from trivial to serious.

Human Growth Hormone (HGH) has an Asp- at position 130. In the example, this form is resolved from the variants with isoAsp- and succinimide at position 130. Resolution from deamidated variants is even better. HGH also undergoes nonenzymatic loss of the first tow residues. This desPhe-Pro- form is well separated from HGH, as are its corresponding variants.

Data courtesy of Benny Welinder, Novo Nordisk A/S)

Recombinant Protein Variant Analysis
-Human Growth Hormone after 6 days at 37°-



Column: PolyCAT A 1000Å
Gradient: 130-145 mM NH_4-acetate, pH 4.0, with 40% MeCN; 30°

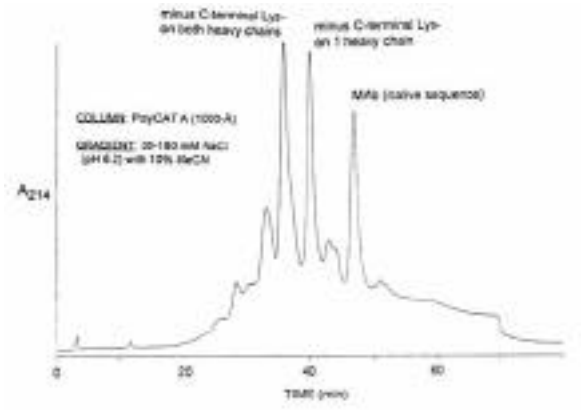
The Nest Group, Inc. TEL# 800-347-6378 or TEL# 508-481-6223 FAX# 508-485-5736
45 Valley Road, Southboro, MA 01772 WWW URL http://world.std.com/~nestgrp email: nestgrp@world.std.com

Monoclonal Antibody Variant Analysis

Monoclonal antibodies (MAb's) form variants too. A common deletion is the first residue at the C-terminus of the heavy chain. That residue is always Lys- or Arg-. In the example below, the third major peak is the intact MAb. The second major peak is the variant minus the terminal Lys- or Arg- from one heavy chin. The first major peak is the terminal residues from both heavy chains. The minor peaks represent more extensive deletions or other variations such as oxidation and the like.

Many MAb's are glycosylated, with the branches terminating in sialic acid. The number of sialic acid residues can vary. The activity of the MAb is sometimes crucially dependent on the degree of glycosylation and sialylation. The conditions used to resolve sequence variants can also be used to separate sialylation variants. Generally, the best conditions to use are cation-exchange on PolyCAT A (1000Å or 1500Å pore diameter), with a shallow gradient of increasing pH and salt concentration. The optimal pH must be determined anew with each MAb.

"Pure" Monoclonal Antibody on PolyCAT A



Ordering Information:

Description	Part Number	Price
PolyAspartic Acid WCX column, 200 x 4.6mm (5µ, 1000Å PolyCAT A)	P204CT0510	$510.00
PolyAspartic Acid WCX column, 200 x 2.1mm (5µ, 1000Å PolyCAT A)	P202CT0510	$510.00
PolyAspartic Acid WCX column, 100 x 4.6mm (5µ, 1000Å PolyCAT A)	P104CT0510	$430.00
PolyAspartic Acid WCX column, 100 x 2.1mm (5µ, 1000Å PolyCAT A)	P102CT0510	$430.00
PolyAspartic Acid WCX column, 200 x 4.6mm (5µ, 1500Å PolyCAT A)	P204CT0515	$510.00
PolyAspartic Acid WCX column, 200 x 2.1mm (5µ, 1500Å PolyCAT A)	P202CT0515	$510.00

Other column dimensions are available from 0.5 to 41.4mmID as well as pore diameters of 200Å and 300Å.
**For proteins and polypeptides above 15KD, selectivity is usually better with 1000Å than with 300Å.*

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