### **Protein Purification** Affinity purification - Coupling







#### Covalently Immobilized Ligand (attached by amide bond and long spacer arm)

Thermo Scientific Pierce CarboxyLink support immobilisation chemistry.

# CarboxyLink immobilisation kits and coupling resins, Thermo Scientific Pierce

## Thermo



GE

NEV

Immobilise peptides via carboxyl groups to create an affinity column.

- CarboxyLink coupling resin DADPA-activated crosslinked 4% beaded agarose (or DADPA-activated UltraLink™ support, a beaded polyacrylamide resin)
- Efficient immobilisation couple 1 to 2mg of peptide per mL of resin (CarboxyLink agarose resin activated with greater than 16µmol amine/mL of resin; DADPA on UltraLink support activated with greater than 40µmol amine/mL of resin)
- Stable linkage immoblisation results in covalent attachment of carboxyl groups by amide bonds, allowing for multiple rounds of affinity purification with one batch of prepared resin
- Flexible and gentle coupling conditions immobilisation reaction completed in simple MES or other non-amine and non-carboxyl, near-neutral buffer, with or without organic solvent

CarboxyLink<sup>™</sup> resin is crosslinked beaded agarose (or polyacrylamide Thermo Scientific Pierce UltraLink support) that has been activated with diaminodipropylamine (DADPA) to contain long spacer arms, each with a primary amine at the end. When incubated with the resin and the carbodiimide crosslinker EDC (included in the CarboxyLink immobilisation kit), carboxyl-containing molecules become permanently attached to the support by stable amide bonds.

Description	Quantity
<b>CarboxyLink immobilisation kit.</b> Includes: DADPA columns EDC Coupling buffer Wash buffer	Kit
CarboxyLink coupling resin Support: Crosslinked 4% beaded agarose Loading: 16 to 20µmol amino groups/mL of resin	25mL
Carboxylink immobilisation kit with UltraLink resin Support: UltraLink Biosupport Loading: ≥40µmol amino groups/mL of resin Includes: UltraLink DADPA columns EDC Coupling buffer Wash buffer Accessories	Kit
EDC 1-Ethyl-3-[3-dimethylaminopropyl]carbodiimide hydrochloride	5g
BupH MES buffered saline packs	10 pack
	Description CarboxyLink immobilisation kit. Includes: DADPA columns EDC Coupling buffer Wash buffer CarboxyLink coupling resin Support: Crosslinked 4% beaded agarose Loading: 16 to 20µmol amino groups/mL of resin Carboxylink immobilisation kit with UltraLink resin Support: UltraLink Biosupport Loading: ≥40µmol amino groups/mL of resin Includes: UltraLink DADPA columns EDC Coupling buffer Wash buffer Accessories EDC 1-Ethyl-3-[3-dimethylaminopropyl]carbodiimide hydrochloride BupH MES buffered saline packs

#### Technical Specification - Specific

Group to be coupled	-NH <sub>2</sub>
Coupling conditions	pH6.5 to pH9 at 4°C to 25°C
Matrix	Highly cross-linked agarose 6%
Medium	NHS Sepharose High Performance
Substitution	10µmol NHS/mL medium
Spacer arm	6-aminocaproic acid, 10-atom
Particle size, µm	34 (average)
Stability	pH3 to pH12 (ligand dependent)
Volume, µL	600 (sample loading, max.)
Material	Polypropylene barrel and polyethylene frits
Storage	100% isopropanol
Temperature, storage, °C	4 to 30

### **NHS HP SpinTrap**



Enrichment of target proteins.

- Antibody or capturing molecule covalently immobilised via free NH<sub>2</sub> groups
- Simple coupling, carried out near neutral pH
- Fast and flexible protocol for the capture step, with elution conditions formatted for both electrophoresis and LC-MS analysis workflows
- Easy scale-up to HiTrap NHS-activated HP columns
- The kit contains bulk media and empty SpinTrap columns

Includes empty SpinTrap columns and NHS activated Sepharose High Performance medium in bulk to allow for batch coupling. Proteins of interest are captured and enriched via an antibody or other molecule (e.g., protein) with desired affinity coupled to the medium through the primary amine. NHS Sepharose High Performance medium is delivered in 100% isopropanol to protect the active groups.

Catalogue No	Alt. No	Description	Quantity
GZ28903128	28-9031-28	NHS HP SpinTrap	5mL media plus 24 columns