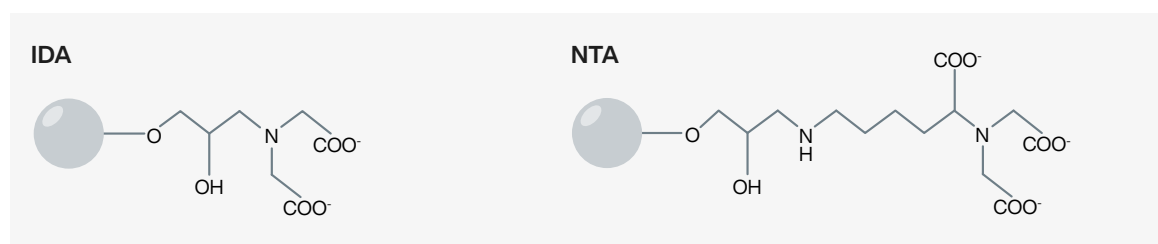


TECHNICAL NOTE

Properties of WorkBeads IMAC resins with focus on buffer compatibilities and pH tolerance (based on RSF data)

The regulatory support files (RSF) provide an invaluable starting point for process development and validation. It is also critical for preparation of standard operating procedures (SOPs), quality control, and as support to clinical and marketing applications to regulatory agencies. Thus, these documents are of utmost importance. However, since they contain large data set including *e.g.* extractables, an overview of important storage conditions for the IMAC resins are presented in this short technical note.

There are three different chelating ligands that WorkBeads™ IMAC resins are based on. First WorkBeads IDA (iminodiacetic acid) and WorkBeads NTA (nitrilotriacetic acid) which can be charged or are pre-charged with 4 different metals (Ni^{2+} , Co^{2+} , Cu^{2+} , Zn^{2+}). Different combinations of ligands and metals give unique selectivities. Pre-charged IDA and NTA-based IMAC resins can be stripped, cleaned and recharged with fresh metal ions.



The third resin is WorkBeads NiIMAC, a nickel pre-charged resin with very low nickel leakage and tolerant against chelating agents (EDTA) and reducing substances (DTT). This resin is optimal for purification of *e.g.* eukaryotic cell feeds often containing these substances.

- WorkBeads IDA
- WorkBeads NTA
- WorkBeads NiIMAC

Buffer compatibilities

The buffer compatibilities were tested for all resins incubated in selected process solutions for one week at ambient temperature, approximately 22°C. The analyses performed were the following: copper binding capacity (CBC) for uncharged IMAC and retention times of His₆-GFP for Ni²⁺-charged IMAC resins.

Uncharged WorkBeads IMAC resins (IDA and NTA) are fully compatible with the following conditions:

- 70% ethanol
- 1 M NaOH
- 50 mM Na₂EDTA, pH 8

The specific solution compatibilities for WorkBeads Ni-NTA, whose Ni²⁺ can be stripped and re-charged and WorkBeads NiMAC that have very stable metal ion bindings, which cannot be stripped or recharged with new metals are shown in tables 1 and 2. Check box (✓) means no difference in performance, i.e. complete compatibility with the specified solution.

Table 1. Ni-NTA

| Process solutions | WorkBeads Ni-NTA |
|----------------------------------|------------------|
| 70% ethanol | ✓ |
| 8 M urea | ✓ |
| 50 mM Na ₂ EDTA, pH 8 | ✓ |
| 10 mM DTT | ✓ |
| 10 mM TCEP | ✓ |
| 20 mM β-mercaptoethanol | ✓ ¹ |
| 500 mM imidazole | ✓ ¹ |

¹ Slight change in retention time for His₆-GFP

Table 2. NiMAC

| Process solutions | WorkBeads NiMAC |
|----------------------------------|-----------------|
| 95% ethanol | ✓ |
| 30% isopropanol | ✓ |
| 6 M guanidine hydrochloride | ✓ |
| 8 M urea | ✓ |
| 20 mM Na ₂ EDTA, pH 8 | ✓ |
| 20 mM DTT, | ✓ |
| 20 mM TCEP | ✓ |
| 20 mM β-mercaptoethanol | ✓ |
| 500 mM imidazole | ✓ |

¹ Slight change in retention time for His₆-GFP

pH tolerance

The operative and storage pH tolerance is another important parameter to consider. The uncharged NTA- and IDA-based resins are more tolerant compared to the pre-charged NTA and IDA-based resins. NiMAC has a broader operative pH range but is intolerant to high pH for longer exposure times.

| | Working range | CIP range |
|---|---------------|----------------------|
| Uncharged WorkBeads NTA/WorkBeads IDA | pH 2–12 | pH 2–12 |
| Pre-charged WorkBeads NTA/WorkBeads IDA | pH 7–9 | pH 2–12 ¹ |
| WorkBeads NiMAC | pH 3–9 | pH 2–14 ² |

¹ The resin gets stripped of metals

² Only for short exposure times; the product is intolerant to high pH for extensive exposure times.

Cleaning-in-place (CIP) conditions

WorkBeads NiMAC: Cleaning with up to 0.5 M NaOH for 15 minutes (keep the contact time as short as possible) followed by 15 minutes distilled H₂O in repeated cycles are recommended if resin gets fouled. 150 mM phosphoric acid can additionally be used prior to NaOH with water washes between. The product should not be stored at high pH overnight.

WorkBeads NTA/ WorkBeads IDA (metal ion stripped resin): Cleaning with 1M NaOH applied by a low reversed flow for 2 hours or overnight is often sufficient to remove heavily fouled resins, otherwise a shorter contact time is enough. Sanitization (reduction of microorganisms) is done by combinations of NaOH and ethanol (e.g. incubation with a mixture of 0.5 M NaOH and 40% ethanol for 3 hours).

- Compatible with commonly used buffers
- pH tolerance varies among the different products

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