

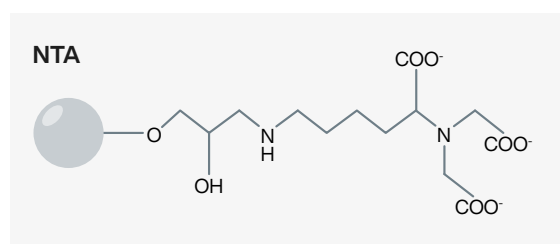
## TECHNICAL NOTE

# 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.

WorkBeads™ IMAC resins are based on NTA (nitrilotriacetic acid) and a proprietary ligand used in WorkBeads NiMAC.

WorkBeads NTA is supplied either in an uncharged form or pre-charged with Ni<sup>2+</sup>. The selectivity of IMAC purification is influenced by the choice of ligand and metal ion and can be custom pre-charged with Co<sup>2+</sup>, Cu<sup>2+</sup>, Zn<sup>2+</sup>. NTA-based resins can be stripped, cleaned, and recharged with fresh metal ions.



WorkBeads NiMAC, 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 NTA
- WorkBeads NiMAC

## 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 His<sub>6</sub>-GFP for Ni<sup>2+</sup>-charged IMAC resins.

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

- 70% ethanol
- 1 M NaOH
- 50 mM Na<sub>2</sub>EDTA, pH 8

The specific solution compatibilities for WorkBeads Ni-NTA, whose Ni<sup>2+</sup> 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 <sub>2</sub> EDTA, pH 8	✓
10 mM DTT	✓
10 mM TCEP	✓
20 mM β-mercaptoethanol	✓ <sup>1</sup>
500 mM imidazole	✓ <sup>1</sup>

<sup>1</sup> Slight change in retention time for His<sub>6</sub>-GFP

Table 2. NiMAC

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

<sup>1</sup> Slight change in retention time for His<sub>6</sub>-GFP

## pH tolerance

The operative and storage pH tolerance is another important parameter to consider. The uncharged NTA-based resins are more tolerant compared to the pre-charged NTA 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	pH 2–12	pH 2–12
Pre-charged WorkBeads NTA	pH 7–9	pH 2–12 <sup>1</sup>
WorkBeads NiMAC	pH 3–9	pH 2–14 <sup>2</sup>

<sup>1</sup> The resin gets stripped of metals

<sup>2</sup> 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 H<sub>2</sub>O 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 (metal ion stripped resin):** Cleaning with 1 M 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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