

## INDIGO-Ni Regeneration Protocol

### **When to apply this protocol:**

Indigo based agarose resins and MagBeads should be washed & regenerated at least after every 5<sup>th</sup> use. Though we recommend to perform this regeneration protocol after each use, if possible.

### **The protocol:**

**Note:** "CV" refers to column volume, i.e. for 1 ml column bed volume use 10 CV = 10 ml of buffer

1. 10 CV H<sub>2</sub>O
2. 10 CV 500 mM NaOH
3. 10 CV H<sub>2</sub>O
4. 10 CV Neutralization buffer  
(150 mM Sodium chloride, 200 mM Na<sub>2</sub>HPO<sub>4</sub> pH 7.0)
5. 10 CV H<sub>2</sub>O
6. 10 CV 20% (v/v) Ethanol, 10 mM sodium acetate, pH 6.5  
(This is the recommended storage buffer for both agarose resin and MagBeads)

### **Side note:**

The INDIGO ligand cannot be stripped like e.g. Ni-NTA. The Nickel ions bind strongly to it. Therefore this protocol does not contain a "reload" with new nickel ions as it the case with NTA regeneration protocol.