# INDIGO-Ni Regeneration Protocol

**When to apply this protocol:**

Indigo based agarose resins and MagBeads should be washed & regenerated at least after every 5$^{th}$ 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 $H_2O$
2. 10 CV 500 mM NaOH
3. 10 CV $H_2O$
4. 10 CV Neutralization buffer
   (150 mM Sodium chloride, 200 mM $Na_2HPO_4$ pH 7.0)
5. 10 CV $H_2O$
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 with 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.