PROTOCOL		
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Nickel Column preparation & maintenance

Preperation

Materials & Equipment

- Novagen Ni-NTA His-Bind Resin (Cat#70666-4)
- MQ water – 25ml
- EtOH 20% - 25ml

Experiment procedure

1. Take a clean glass column (Biorad) and connect the appropriate valve to nozzle – set valve to "close" position.
2. Fix column to a stand and thoroughly vortex Nickle beads.
3. Using a pasteur pipette pour mixed beads onto column till the slurry reaches 5mm above plastic cover.
4. Open valve and let the nickel flowthrough.
5. Fill column with MQ and let it flowthrough to the waste.

Note: If column is to be used immediately, you can load your sample at this point.

6. Close valve and fill column with 20% EtOH (about 25ml); seal column with cap and place it in cool place or at 4°C.

Stripping used column

Materials & Equipment

- Stripping buffer (see protocol #4 for details) – 25ml
- MQ water – 400ml
- Guanidine 6M – 25ml
- Nickel 0.2M – 25ml **(place back at 4°C!)**
- EtOH 20% - 25ml

Note: Unless stated otherwise, all washes are performed at column volume.

Experiment procedure

1. Drain column and wash 3 times with MQ ***(only if column was mounted with EtOH 20%).***
2. Wash column with stripping buffer.
3. Wash column with Guanidine 6M.
4. Wash column 5 times with MQ.
5. Wash column with Nickel 0.2M.
6. Wash column 5 times with MQ.
7. Close valve and load column with EtOH 20% - keep at RT.

Buffer	Ingredients	Volume	MQ
Stripping buffer (25ml)	50mM Tris pH=8	0.5ml	Fill to 25ml
	500mM NaCl	2.5ml	
	50mM EDTA	2.5ml	