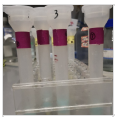

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10 Affinity Chromatography

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1 Works for me dx.doi.org/10.17504/protocols.io.5cgg2tw

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MATERIALS

NAME	CATALOG #	VENDOR
EDTA		
100ml GST Elution Buffer (5mM glutathione, 50mM Tris.HCl (pH8.0))	786-541	G-Biosciences
5 columns Ni-NTA Resin, 1ml Spin Column	786-944	G-Biosciences
GSH-Glo(TM) Glutathione Assay, 50ml	V6912	Promega
Guanidine hydrochloride	GB0242.SIZE.100g	Bio Basic Inc.
double distilled water (ddH2O)		
Ni-binding buffer	/	
Ni-Washing buffer	/	
Ni-Elution buffer	/	
Protein solution that needs to be purified	/	
NiSO4	/	
GST columns	/	
GST-washing buffer	/	
GST-binding buffer	/	

1 **Lysis of the bacteria.**

1. Resuspend the frozen cell paste as best you can in the Lysis Buffer using a 10 mL pipet or whatever means necessary. Let this suspension incubate for 20 minutes at room temperature, or until the suspension becomes turbid and viscous due to release of the bacteria's genomic DNA.

10 ml **00:20:00**

2. Smash the bacteria.
3. Centrifuge at 18,000 rpm in a big rotor for 40 minutes at 4°C. Save the pellet and the supernatent.

18000 rpm **00:40:00** **4 °C**

2 **IMAC**(Immobilized metal ion affinity chromatography)

Procedure

1. Remove the Ni column from the 4°C refrigerator, which contains 20% alcohol. Wash the column with water for 10 minutes. Change to Ni-binding buffer for another 10 minutes and balance the Ni column.

4 °C

2. Add the protein solution to the column, let it flow naturally and bind to the column. Repeat until the medium turns gray (usually twice).
3. Add Ni-Washing buffer several times and let it flow. Take 5ul of wash solution and test with Coomassie Brilliant Blue. Stop washing when

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it doesn't turn blue.

5 µl

4. Add Ni-Elution buffer several times. Check as above.
5. Collect the eluted proteins for further operation. Recycle columns: Wash with 0.2mM EDTA , let stand for 10 min then drain. Wash with 6M Guanidine hydrochloride, let stand for 10 min then drain. Wash with ddH_2O for three times. Fill up with $NiSO_4$, place on a shaker overnight at 4°C.

4 °C

GSTAffinity chromatography of glutathione transferas
Procedure
1. Remove the GST column from the 4°C refrigerator. Wash the column with GST-binding buffer for 10 minutes to balance the GST column.

4 °C **00:10:00**

2. Add the protein solution to the column, let it flow naturally and bind to the column.
3. Add GST-Washing buffer several times and let it flow. Take 5µl of wash solution and test with Coomassie Brilliant Blue. Stop washing when it doesn't turn blue.

5 µl

4. Add GST-Elution buffer several times. Check as above.
5. Collect the eluted proteins for further operation. Recycle columns: Wash with GST-Elution buffer until Coomassie Brilliant Blue doesn't turn blue when tested. Wash with 6M Guanidine hydrochloride , let stand for 10 min then drain. Wash with ddH_2O for three times.

00:10:00

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