FG winds

Issue 1
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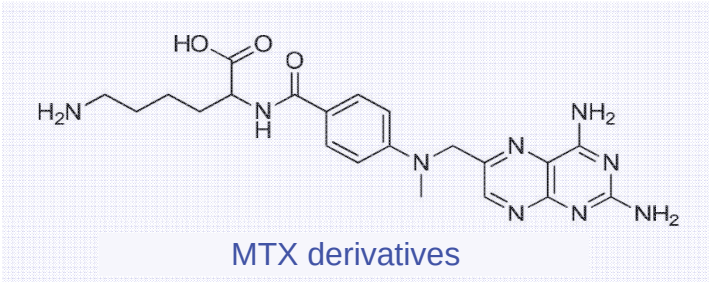
Isolation of drug target protein

Summary

Chemical Biology is known as new biological approach for analysis of the interaction between small molecules (chemicals) and target molecules in the cells.
FG beads are the best tool for this purpose. We here introduce how to immobilize chemical on the FG beads and show the result with competitors.

Experimental Information

MTX (Methotrexate) is an anti metabolite and anti folate drug. It is used in treatment of cancer and autoimmune diseases. We immobilized MTX derivatives on FG beads to capture target molecules in HeLa cell extracts.



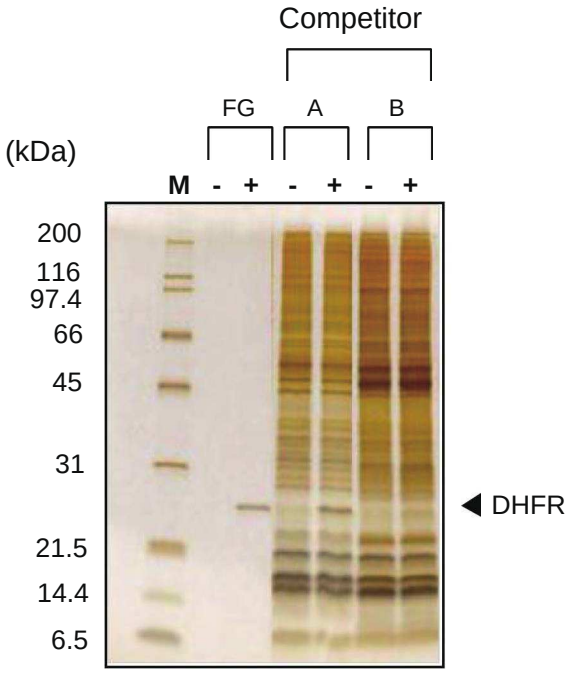
MTX derivatives

Result

1. FG beads captured DFHR which is an enzyme that reduces dihydrofolic acid to tetrahydrofolic acid.
This result was quite reasonable to follow the publishments that the target of MTX is DHFR.

2. We also compared FG beads with other commercially available magnetic beads.
We confirmed FG beads have
* extremely lower background.
* larger amount of captured DHFR.

It suggests FG beads are useful for the analysis of the interaction between small molecule and target molecule.



Immobilization of MTX on FG beads and Isolation of target protein

Materials and method

■Materials

1. MTX derivatives
2. NHS beads (cat#TAS8848N1141 and competitor A)
COOH beads (competitor B)
3. HeLa cell extracts (cytosolic fraction) --3mg/ml
4. Binding &Washing Buffer (20mM HEPES-NaOH(pH7.9), 100mM KCl, 1mM $MgCl_2$, 0.2mM $CaCl_2$, 0.2mM EDTA, 10%(v/v) glycerol, 0.1% NP-40, 1mM DTT, 0.2mM PMSF)
5. Elution Buffer (0.0625M Tris-HCl (pH6.8), 0.005% BPB, 2% SDS, 10% glycerol, 5% 2-mercapto ethanol)

■Methods 1 (Immobilization)

1. Apply

FG beads
0.5mg NHS beads and 0.1mM MTX in 100ul DMF
0.5mg NHS beads and NO MTX in 100ul DMF (Negative Control)

Competitor A
0.5mg NHS beads and 0.1mM MTX in 100ul 50mM HEPES pH7.0
0.5mg NHS beads and NO MTX in 100ul 50mM HEPES pH7.0 (Negative Control)

Competitor B
0.5mg COOH beads, 0.1mM MTX and EDC-HCl in 100ul 15mM MES pH6.0
0.5mg COOH beads, NO MTX and EDC-HCl in 100ul 15mM MES pH6.0 (Negative Control)

2. Reaction

1) Immobilization

FG beads	70min	at r.t.
Competitor A	70min	at r.t.
Competitor B	overnight	at r.t.

＊ Recommended conditions of each beads.

2) Masking
Inactivate unreacted NHS or COOH according to recommended conditions of each beads.

■Methods 2 (Affinity purification)

1. Wash
Wash beads with washing buffer 3 times at 4ºC (on ice).
2. Add sample solution
Add 200ul HeLa cell extracts to beads.
3. Reaction
Mix for 120min at 4ºC.
4. Wash
Remove sample solution.
Wash beads with washing buffer 3 times at 4ºC (on ice).

■Methods 3 (Elution)

Add 40ul elution buffer and resuspend beads.
Boil for 5min at 98ºC and remove beads.
Analyze the samples by SDS-PAGE and silver staining.