Chemical Biology Services

www.FGbeads.com

Target Purification / Target Fishing

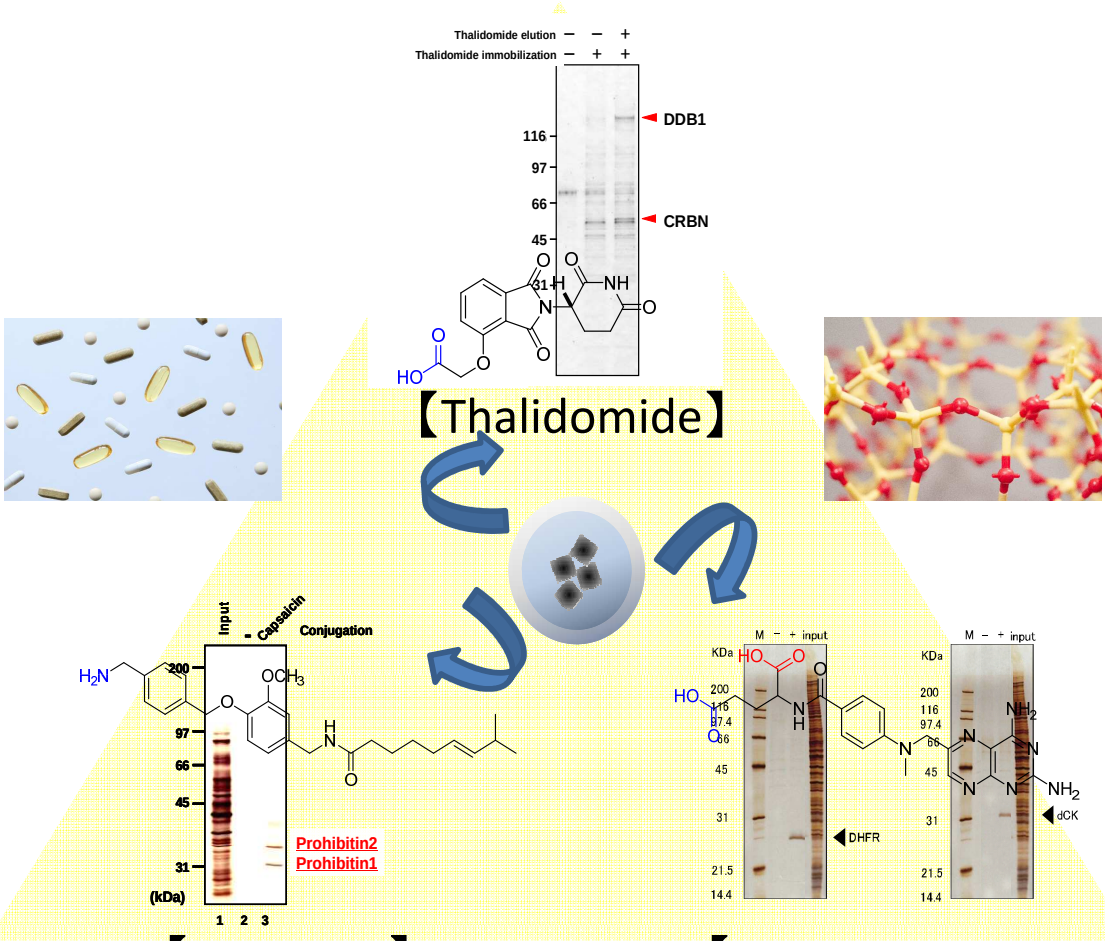
*** How do you usually confirm that your compound does not bind to unwanted target in cells?**

***Would you like to discover molecular target or new function of your compound?**

Recently, chemical Biology is becoming very popular to find out molecular targets for existing compounds, natural small molecules, and side-effect's research.
There are many existing drugs(small compounds), still not identified molecular target(s) and many researches to find new drug candidates from natural compounds library. However, it is still hard to identify endogenous target protein(s) binding with compound(s).
On the other hand, it is also difficult to purify off-target protein(s) which may cause side-effects against small compound in current biochemical approaches. There are many approaches for this dilemma such as HPLC analysis.
We introduce our services for this approach using our high performance magnetic nanoparticles (FG beads®). There are various FG beads® line-ups with different functional group to meet the best selection depends on your compound's SAR.*
Our services consists on 1. consultation for your compound, 2. design and synthesize the compound, 3. immobilization of your compound on the FG beads®, 4. reaction with cell extracts, 5. find specific target protein, 6. identify target protein(s) by LC-MS (optional).
*We might ask you to re-synthesize compound adding new functional group when no right functional group for immobilization by SAR study.

New Approach for chemical biology

FG beads® gives you new idea to identify endogenous target protein against small molecule compound and helps for the purification of it. We offer the services to purify target protein(s), bound to your compound.



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Service Flow

We ask you to accept our contract to proceed this service. Please download our "Service contract" from www.FGbeads.com/contract/en and sign /email back it to info@tamagawaseiki.co.jp

1. Consult, Design, and Synthesize the compound for the immobilization on certain FG beads®.

Based on the disclosure of SAR study with your compound, we will find the best way which FG beads® is suitable for your compound and whether your compound is necessary to modify/add extra functional group.

No need the modification

↓

Go "2" experiment

If necessary additional functional group

↓

Synthesize compound with additional functional group by yourself or by us

↓

Go "2" experiment

2. Immobilize the compound on certain FG beads® and confirm the immobilization.

Apply four(4) different dose e.g.(0, 3, 10, 30mM) of compound to FG beads® according to our standard protocol for immobilization.
Check* the amount of compound by HPLC, actually immobilized on beads.
----- It is impossible to measure the amount of compound on the beads when we use beads other than NHS FG beads®.
In that case, we confirm dose dependency by binding experiment with HeLa cell extracts.

3. Affinity purification of binding protein(s)

Reaction immobilized beads with cell extracts provided by customer

•We use below experimental matrix and condition

-- Experimental Matrix(Example)

	Conc.of compound
1	0mM
2	3mM
3	10mM
4	30mM

React !!

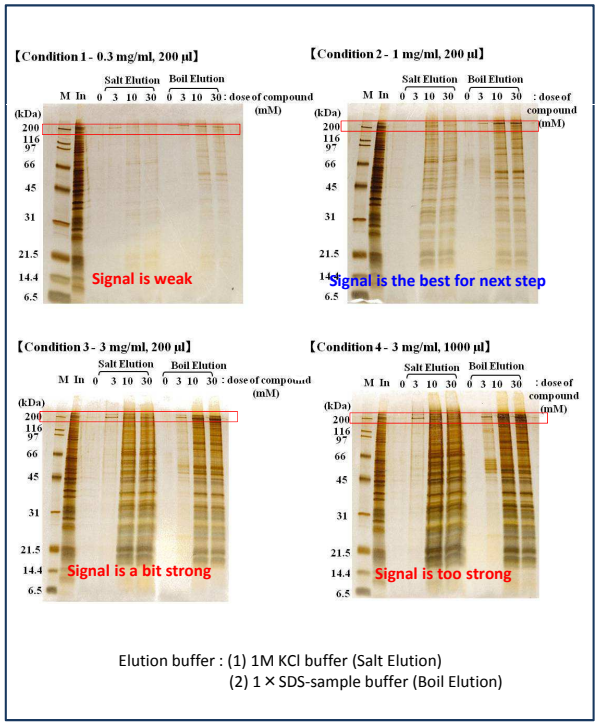
condition	Conc. of cell extracts & Vol.
1	0.3mg/ml x 200ul
2	1mg/ml x 200ul
3	3mg/ml x 200ul
4	3mg/ml x 1000ul

-- Experimental condition

- Wash & Binding buffer : 20 mM HEPES-NaOH(pH7.9) , 100 mM KCl, 1 mM $MgCl_2$, 0.2 mM $CaCl_2$,0.2 mM EDTA, 10% glycerol, 0.1 % NP-40, 1 mM DTT, 0.2 mM PMSF
- Cell extracts : Provided by customer
- Reaction time & temp. : 4 hours at 4℃

Once immobilized beads was reacted with cell extracts, we elute the bound proteins with salt and boiling and checking by SDS-PAGE with silver staining. Then we confirm which condition was the best for next experiment as indicated the example (right side).

If the best condition was not found in matrix combination, we will ask you to consider optionally trying different buffer condition (lower or higher salt conc.).



Elution buffer : (1) 1M KCl buffer (Salt Elution)
(2) 1 × SDS-sample buffer (Boil Elution)

4. Find specific target protein(s)

We basically use drug elution method to find protein(s) specifically bound to the drug. As an option, drug competition assay is also available.
*If drug elution did not work well to find specific target(s), we might suggest to go next step.
----- In case of the associated protein is similar molecular weight with target protein, it could be difficult to find out target by drug elution or competition assay.

5. Identification of target protein(s) ---Optional

Analysis by Mass-Spec. is useful to identify target proteins. We will outsource this analysis to third party OR will also provide you the samples for your analysis.