

Frequently Asked Questions about the FKBP Destabilizing Domains (DDs)

1) How do I propagate the plasmids?

All plasmids are pBMN retroviral (MMLV-derived) plasmids containing the ampicillin resistance gene. We typically propagate and clone in TOP10 cells at 30 °C to prevent recombination, which we have seen when cloning at 37 °C. You should be able to propagate these plasmids in any garden-variety *E. coli* "cloning strain" such as DH5 α , but be aware that recombination can be a problem. These plasmids can be used to make both ecotropic and amphotropic virus, depending on the packaging cell line or helper plasmid used to make virus.

2) What sites are available for cloning?

When using an N-terminal FKBP DD, the protein of interest can be cloned into the vector using EcoRI as the 5'-site and either XhoI, NotI, SacII, or SnaB1 as the 3'-site. When using a C-terminal FKBP, the protein of interest must be cloned using BamHI as the 5'-site and EcoRI as the 3'-site. The full sequences of the plasmids are available as PDF files on our website at: <http://wandless.stanford.edu/Plasmidseq.htm>

3) Can you recommend a good anti-FKBP12 antibody?

Yes, at least we can recommend two not-so-bad antibodies. Affinity Bioreagents (Golden, Colorado, bioreagents.com, 800-527-4535) catalog PA1-026A. Product info claims that this antibody is good for IHC, IP and Western. It's a rabbit polyclonal against a 13-residue N-terminal FKBP epitope. More recently we have used a monoclonal from BD Pharmingen (<http://wwwbdbiosciences.com/>) catalog 610808. It has also been pretty good for Westerns of the L106P DD.

4) Where can I get Shield-1 and/or related plasmids?

We have shipped more than 500 samples to over 300 different labs. As you might imagine, this has taken quite a bit of effort. There are also two commercial suppliers as of December 2008. Clontech sells Shield-1 as well as other related reagents under the "ProteoTuner" name. A contract synthesis company called Cheminpharma (New Haven, CT) also sells Shield-1.

5) How do I formulate Shield-1 (Shld1)?

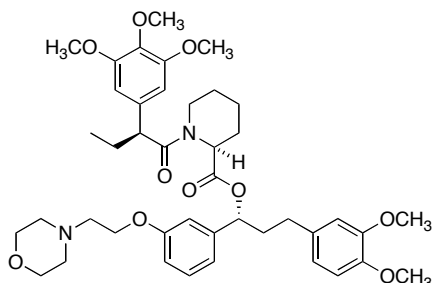
The molecular weight of Shld1 is 748.91 (C₄₂H₅₆N₂O₁₀). We typically make a 1 mM stock in absolute (200 proof) ethanol, which we store at -20 °C. This stock is good for at least 6 months, probably longer. We use this as a 1000 \times stock, which we dilute directly into cell culture media for each experiment, giving a final concentration of 1 μ M Shld1 (or whatever is required). You may be able to use less Shld1, based on the functional threshold of your protein of interest, which can be determined experimentally.

6) Can I just make a dilution of Shield-1 into media and store at 4 °C?

We don't know. We've never tried this before, and we're not sure how stable the ligand will be for extended periods of time in aqueous solution. If you decide to give it a try, please let us know how it works.

7) Help!!! My vial of Shld1 is empty – the FedEx guy must have taken it!

Relax. Shield-1 is a clear, colorless oil. Go back to step (4) to make a stock and then test it using one of the plasmids we sent encoding YFP. You should see Shld1-dependent YFP by immunofluorescence, Western blot, or flow cytometry.

**Shield-1****8) How soluble is Shield-1 in aqueous solutions?**

Don't expect this compound to be very soluble in water. The highest concentration we've worked with in aqueous solution is 250 μM (1% EtOH). The solution looked a bit cloudy, but we still observed Shld1-dependent activity for our protein of interest. Other than that, the highest concentration we've used in a cell-based experiment is 3 μM , with a 0.3% final EtOH concentration (a 1:300 dilution of a 1 mM stock). Keep in mind that the presence of EtOH may affect the outcome of your experiment, so plan your controls accordingly.

9) Does Shield-1 work in mice? What are its pharmacokinetics?

We have done some experiments demonstrating that Shield-1 is active in mice when delivered intravenously or i.p. See Banaszynski et al. *Nature Medicine* (2008) **14**, 1123-1127.

10) Can I give the plasmids and Shield-1 to my family, friends, casual acquaintances?

Go crazy. Share these reagents with anyone you care to. We do not require MTAs for academic labs. We will try to send the Shield-1 stabilizing ligand to anyone who requests it (as long as they pay for shipping).

11) Does this DD system work in other cell types or organisms?

Yes. Two papers in the December 2007 issue of *Nature Methods* demonstrated that the FKBP-derived DD system regulates protein stability in the apicomplexan parasites, *Toxoplasma gondii* and *Plasmodium falciparum*. We have received anecdotal evidence from other DD users that the system works in zebrafish, *Xenopus*, *Ostreococcus*, and *C. elegans*, but no papers have been published in these organisms to our knowledge.

FAQ sheet last updated: 12/4/08