Fradditive™ Solubility Screens

Application
Fradditive™ screens may be used at all stages of protein production including lysis, refolding, purification, concentration and crystallization.

Physical design
- The library contains a total of 96 solutions
- Library is designed to be used at a 1:10 dilution for a 1mM final concentration. Titrations may be used to sample additional concentrations.

Library preparation
- We recommend that the plate be stored at 4deg C
- We recommend centrifuging the plate before use to ensure all compound is at the bottom of the well. Plates are centrifuged before shipment
- Please remove lid carefully to ensure compound remains in the wells
- The plate is now ready for screening

Performing a Fradditive™ screen
- We recommend testing on small scale either in Eppendorf tubes or deep well blocks. You may choose your endpoint based upon the step of purification you are working. Some example protocols are provided below. You may choose to modify to better fit your particular protein or assay.

Sample lysis protocol to solubilize and stabilize protein from pellet:
- Aliquot 500µl of culture to each well of a 96 well deep block
- Centrifuge and decant the supernatant
- Freeze plate and store at -80 until ready to use
- Prepare your standard lysis buffer including 0.1% weight/volume lysozyme and benzonase 25units/ml (and protease inhibitors)
- Thaw pellets and pipet 450µl of lysis buffer into each well
- Pipet 50µl of Fradditive™ into each well
- Mix at room temperature for 1 hour
- Centrifuge plate to remove cell debris and take samples for analysis by SDS-PAGE
- Include untreated samples of supernatant and pellet as positive controls
- Note that for GFP tagged proteins, concentration of soluble protein may be measured directly

Sample protein solubility/re-folding screen:
- If protein is soluble only at low concentration or has been isolated from inclusion bodies, conditions may be identified where properly folded protein is stable.
- In both cases, prepare your starting buffer condition and pipet 450µl in a 96 deep well block
- Pipet 50µl of Fradditive™ into each well
- For a refolding screen, dilute purified protein from inclusion bodies into each of the Fradditive™ wells and allow to incubate ~3-4hr at RT or overnight at 4deg
- For protein solubility, dilute protein into each of the Fradditive™ wells and allow to incubate overnight at 4 deg
- Protein may be filtered through filter plates such as Pall Acro™-prep which are also available as concentrators
- Depending on the concentration of protein, protein may be further concentrated in 96 well format using Pall Acro™-prep plates and the actual protein concentration compared with the theoretical to calculate a protein yield

Sample protein stability/crystallization screen:

- Fradditive™ may also stabilize the protein in solution for crystallization studies. This may be analyzed by Differential Scanning Fluorimetry (DSF) observation of protein melting temperature. It may also be analyzed by direct observation in crystallization trays.
- To test stability by DSF, we recommend a 1:10 dilution for the starting condition. We typically conduct DSF screens at 2µl protein and 5X SYPRO orange although conditions should be optimized for each protein being tested. Solutions may be prepared in a 96 well plate and the plate centrifuged before placing in the real-time PCR machine. Conditions that stabilize the protein may be incorporated into the purification protocol and/or tested in crystallization experiments.
- Fradditive™ effect on protein solubility may be assessed in vapor diffusion experiments by placing the protein-Fradditive™ mix over high salt to dehydrate the protein or incorporated into a known crystallization condition to improve crystals. Fradditive™ screens are formatted to permit incorporation into routine crystallization additive analysis.

For technical support, please contact erika@zenobiatherapeutics.com