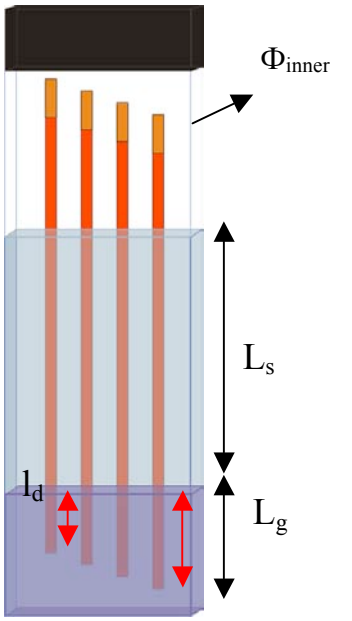
PROTOCOL 2: CD using empty GCB-Domino

Counterdiffusion in the GAME configuration.

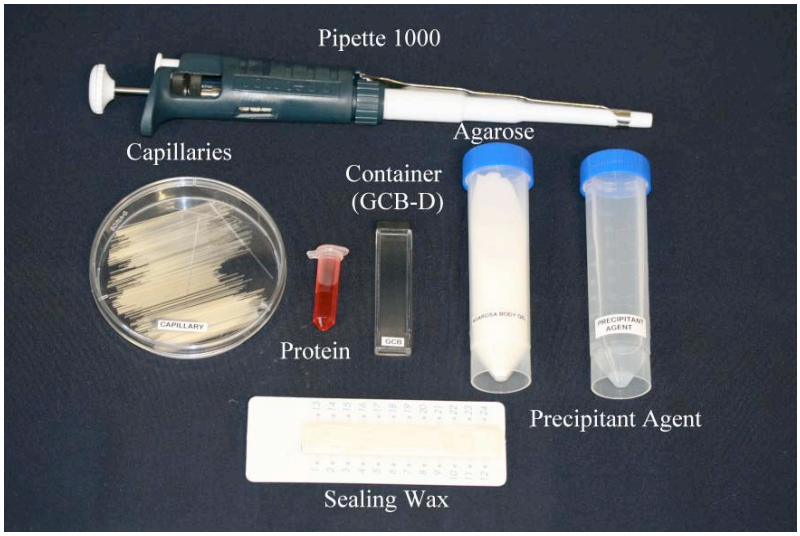
The Gel Acupuncture Method (GAME) can be implemented in any container that can be further close avoiding evaporation.

The GCB-Domino (Triana S&T) is flat container that allows an easy implementation of the experiment, reduce the consumption of reactants and facilitate the observation.



As in any other crystallization experiment there are a number of variables. In counterdiffusion experiments it can be varied the

1. protein concentration
2. salt concentration
3. capillary inner diameter (Φ_{inner})
   Φ_{inner} = 0.1 mm (for screening)
   Φ_{inner} = 0.1 or 0.2 mm (for crystal improvement)
4. punctuation depth (l_d)
   l_d = 5 mm
5. and value and ratio L_s:L_g



Set-up-tools

How to prepare the macromolecular solution

Prepare your macromolecular solution as pure as possible and use it after microfiltration. We recommend the use of a protein concentration of 5 to 10 mg/ml preferentially in water or alternatively in a buffered solution at buffer concentration smaller than 50mM. Initially, the sample should be free of any unnecessary additives in order to observe the effect of the precipitants in the kits.

Note:

We recommend testing two protein concentrations per protein (two capillaries in each box).

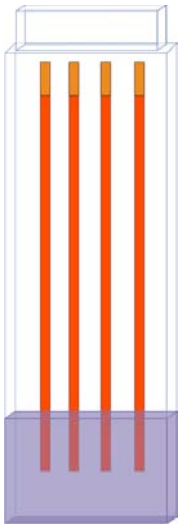
1. GEL LAYER

There are two way to prepare the agarose sol; in a water-heating bath or using a microwave.

1.1. In both cases add the agarose powder to the appropriated volume (5 ml) of the buffer solution to a final agarose concentration of 1% (*w/v*).
1.2. Heat the mixture to boiling until the agarose solution becomes transparent.
1.3. Poor the agarose sol in the GCB and let it gel at room temperature.

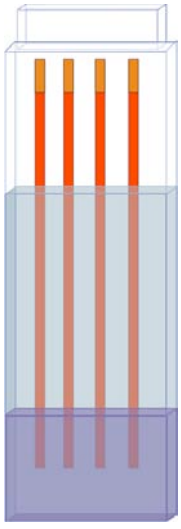
Note: Agarose sol should contain any other additive present in your protein solution.

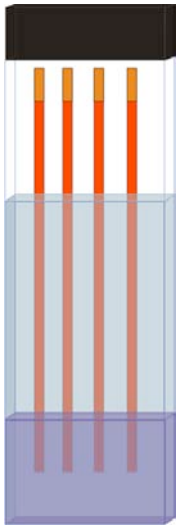
2. FILLING THE CAPILLARIES



2.1. Introduce one end of the capillary into the protein solution. The solution will flow up by capillary force.
2.2. Once it reaches almost the top of the capillary, remove it from the solution (you will see that the solution remains inside the capillary).
2.3. Seal the upper end of the capillary with a small amount of wax.
2.4. Puncture the capillary into the gel layer, typically 5 mm.
Repeat the procedure with the next capillary.

3. PRECIPITANT LAYER

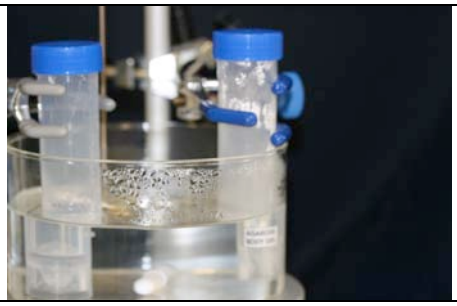
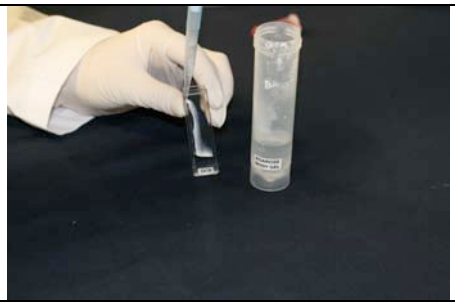




3. Pour the precipitant agent solution.
- For screening we recommend the **Counterdiffusion Screening Solutions (CSS)®**

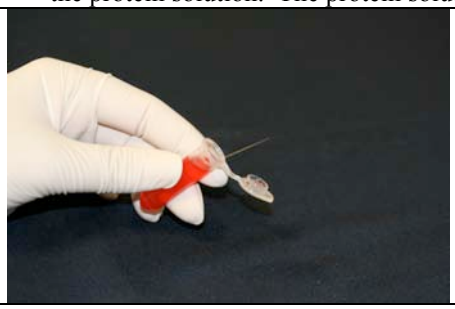
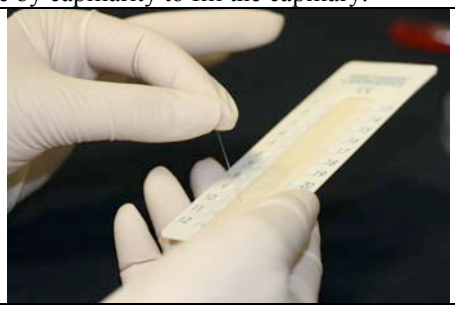
Close the box and sealed with parafilm.

STEP BY STEP

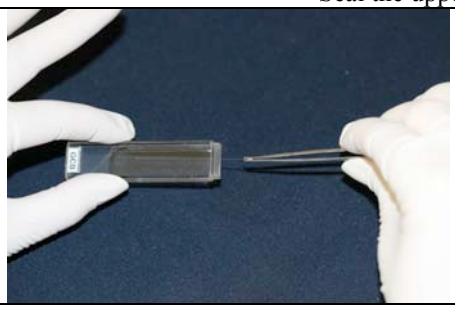
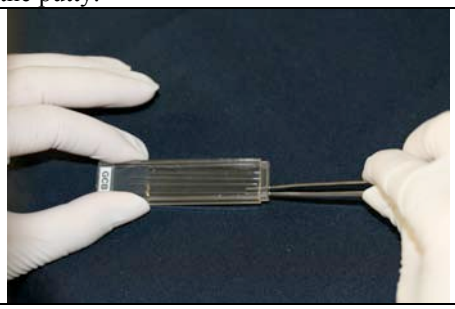



Prepare the agarose sol in the desired buffer and fill the reservoir to the desired high.

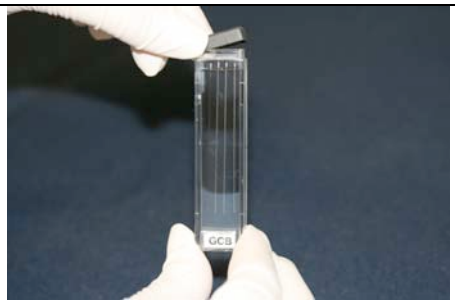

Mark the high of agarose layer (Lg) and pour the sol. Let it cool down (10 min.). Dip one capillary into the protein solution. The protein solution will rise by capillarity to fill the capillary.

Seal the upper end with the putty.

Dip the filled capillary into the gel layer to the desired depth. Repeat the sequence with other capillaries filled with your protein solution. Here you can use a different concentration.

Add the precipitant solution to the desired high (Ls). Close the reservoir to avoid evaporation.