Hydra Culturing Protocol

Hydra Medium

Hydra Medium (HM) is used to culture *Hydra*.

Hydra medium is made from two stock solutions:

Stock Solution 1 (1000X)
42.18 g $CaCl_2$ x 2H2O
Up to 1 L Milli-Q Water
Filter Sterilize

Stock Solution 2 (100X)
8.116 g MgSO4 X 7H2O
4.238 g NaHCO3
1.0985 g K2CO3
Up to 1 L Milli-Q Water
Filter Sterilize

20 L *Hydra* Medium
20 mL of Stock solution 1
200 mL of Stock solution 2
Up to 20 L with Milli-Q Water
Stir on stir plate for 45 min

Feeding

Hydra are fed with brine shrimp (*Artemia*)

Materials

San Francisco strain of brine shrimp (*Artemia*):
https://www.brineshrimpdirect.com/?q=san+francisco+strain

Brine shrimp hatchery cone: https://www.brineshrimpdirect.com/large-hatchery-cone-stand-175-oz-bse

Brine Shrimp Strainer: https://www.brineshrimpdirect.com/brine-shrimp-net

Hatching Brine Shrimp

In a brine shrimp hatchery cone, add the following:

37.5 g of non-iodized Morton salt
0.5 g Sodium bicarbonate
1.5 L Milli-Q water
3 scoops of *Artemia* eggs (scoop comes in the container)
Bubble with aeration pump for 2 days.

Harvesting brine shrimp

When they are ready to be harvested, cover the top of the cone with an ice bucket and wait 5-10 minutes until the brine shrimp aggregate at the bottom of the cone (they swim toward the light). Open the tap and collect them in a strainer and rinse with HM. Move the brine shrimp to a dish with HM.

To separate the brine shrimp from the unhatched eggs: Tilt the dish by placing one side on a sharpie or something of similar size. Point a light toward the raised side of the dish. Brine shrimp swim towards light and unhatched eggs fall to the lower side of the dish.

Feeding the *Hydra*

Add necessary amount (depending on size of culture) of *Artemia* that have been freshly cleaned in *Hydra* medium to your *Hydra* dish

Allow the *Hydra* to feed for a minimum of 2 hours before cleaning

Cleaning

Cleaning *Hydra* by hand (smaller cultures)

Obtain a clean dish to move the *Hydra* into

Remove *Hydra* from dirty plate and transfer to a clean dish filled with HM using a glass pipette (Fisherbrand cat#13-678-6A).

IMPORTANT: Use a NEW glass Pasteur pipette for each *Hydra* line. (Fisherbrand cat#13-678-6A). Immediately throw used glass pipettes away in the glass waste to avoid contamination.

Cleaning *Hydra* using strainers (larger cultures)

Carefully pour floating *Hydra* through the strainer, catching liquid into a wash bowl in case some *Hydra* go through the strainer.

Using a wash bottle filled with HM, spray *Hydra* stuck to the dish to dislodge them. Pour *Hydra* into the strainer. *Hydra* can also be dislodged using a gloved hand.

Rinse the *Hydra* in the strainer with HM from the wash bottle until *Artemia* are completely absent.

Fill a new dish (or wash the used dish) with HM.

Use a wash bottle to dislodge the *Hydra* from the strainer into the dish with fresh HM.

Important Tips

Our strainers are tea strainers from Germany, which we get from an “inside” connection. Contact us if you’d like to obtain some special German strainers.

To avoid contamination, we use separate strainers and wash bowls for every strain/transgenic line.

Visually inspect newly washed cultures for large debris and cuticle embryos as these do not pass through the strainer. Remove all debris and cuticle embryos.

If routinely using a strainer for cleaning a line, make sure to manually hand wash your dish once every two weeks in order to ensure that debris and cuticle embryos do not build up. If the *Hydra* line is too large to wash manually, make sure and follow the next tip.

Periodically check the identity of transgenic lines under the fluorescent dissection scope. For cultures that produce a high volume of eggs, occasionally re-start them from the back-up stocks to make sure they are pure; particularly non-transgenic lines that cannot be confirmed visually. Back-up stocks should always be cleaned by hand to ensure purity.