Hybridoma Cell culture with Petaka-HOT (High Oxygen Turnover, orange port)

Production of Monoclonal Antibodies

Petaka-HOT allows culturing non adherent cells such as myeloma and hybridoma cells.

For this application Petaka HOT is used together with the centrifuge and the stands which maintain the Petakas in the required position during incubation to help the cells to be maintained in semi-suspension..

Periodically a centrifugation will concentrate the cells in one of the corners (see centrifugation methods) and segregate the cells and supernatant, allowing Monoclonal Antibodies collection and leaving the cells for further growth and Monoclonal Antibodies production.

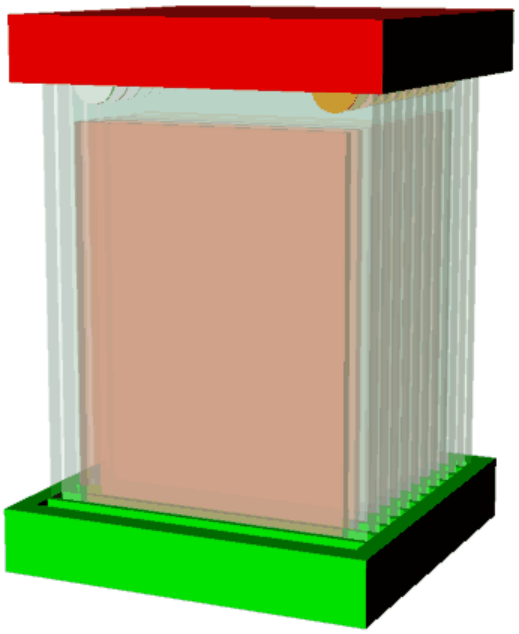
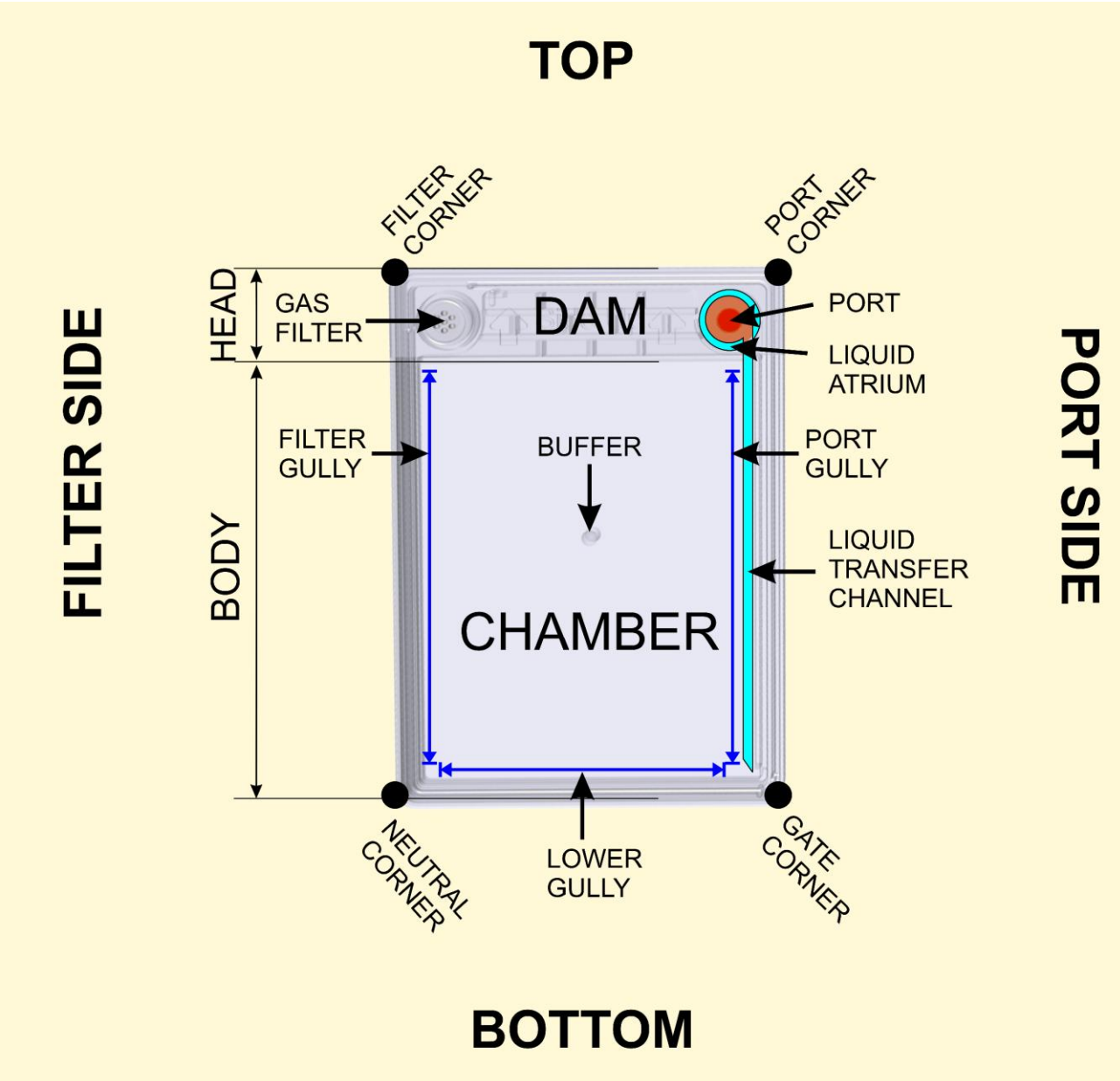


Figure 1.- Set of Petakas bound together with a couple of stands

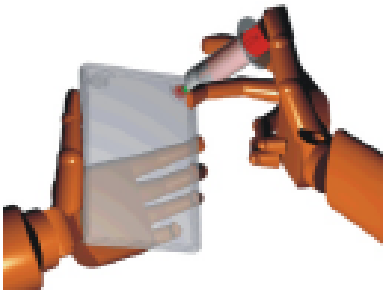
Each set of Petakas, bound with two stands, may Contain up to 275 mL of supernatant which can be collected free of cells by direct centrifugation of the Petakas.

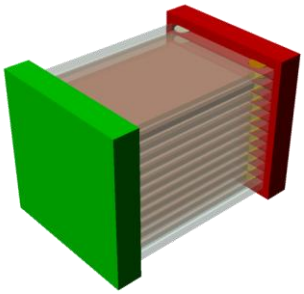
PETAKA ANATOMY AND NOMENCLATOR



PROTOCOL

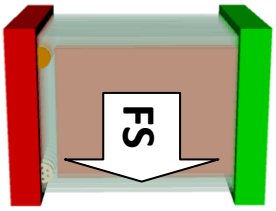
1.- Culture 1M or 2M Hybridoma Cells in Petaka-HOT, and fill it with the selected **hybridoma special media**

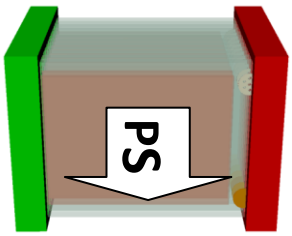




2.- After cell injection, mount the Petakas in a "set" with stands, and live the set in the incubator in horizontal position for 12 hours .

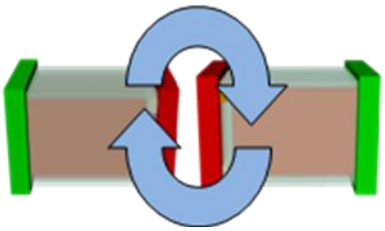
3.- Turn the set 90 degrees to rest the Petakas on the filter side, and incubate for 12 hours in this position. Cells will slowly precipitate to the **filter side (FS)**





4.- Turn the set over (180 degrees) to rest the Petakas on the port side, and incubate for 12 hours in this position. Cells will slowly precipitate to the bottom on the **port side (PS)**

5.- Repeat operations 3 and 4 every 12 hours until the end of the production period (Plateau in Cell growth and Monoclonal Antibodies secretion).



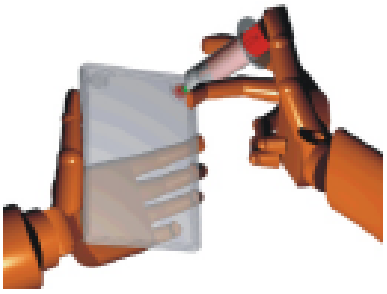
6.- To harvest the supernatant, place the Petakas in the centrifuge buckets with the filter in the uppermost position.

Centrifuge at 1500 rpm for 5 min.

7.- After centrifugation the cell pellet will be formed in the Neutral Corner of Petaka

8.- Completely withdraw the media, avoiding the cell pellet disruption
This contain the Monoclonal Antibodies

9.- Refill Petaka with the selected **hybridoma special media, and resuspend the hybridoma cells.**



10.- Repeat steps 3 to 9 as long the hybridoma continues producing Monoclonal Antibodies