**Submission ID #: 61161**
**Scriptwriter Name: Bridget Colvin**
**Project Page Link:** http://www.jove.com/files_upload.php?src=18654153

# Title: Primary Clarification of CHO Harvested Cell Culture Fluid Using an Acoustic Separator

**Authors and Affiliations: Jin Sung Hong[1], Nicole Azer[1], Cyrus Agarabi[1], and Erica J. Fratz-Berilla[1]**

[1]U.S. Food and Drug Administration, Center for Drug Evaluation and Research, Office of Product Quality, Office of Biotechnology Products, Division of Biotechnology Review and Research II

**Corresponding Author:**
Erica J. Fratz-Berilla
Erica.berilla@fda.hhs.gov

**Co-authors:**
jinsung.hong@fda.hhs.gov
nicole.azer@fda.hhs.gov
cyrus.agarabi@fda.hhs.gov

 

# Author Questionnaire

**1. Microscopy**: Does your protocol involve video microscopy, such as filming a complex dissection or microinjection technique? **N**

**2. Software:** Does the part of your protocol being filmed demonstrate software usage? **Y**

*Videographer: Please film the screen for all SCREEN shots, it was not possible to acquire all screen capture videos*

**3. Filming location:** Will the filming need to take place in multiple locations (greater than walking distance)? **N**

**Protocol Length**
Number of Shots: **45**

# Introduction

## 1. Introductory Interview Statements

**REQUIRED:**

1.1. **Jin Sung Hong**: Our protocol demonstrates how to use a bench-scale acoustic wave separator for the primary separation of CHO cells from harvested culture fluid containing a model monoclonal antibody **[1]**.

1.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested b-roll: Lab media: Figure 8*

**REQUIRED:**

1.2. **Jin Sung Hong**: Acoustic wave separation provides some advantages compared to traditional primary clarification methods like centrifugation and filtration, such as a small footprint, low contamination risk, and reduced filter fouling risk **[1]**.

1.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera

**OPTIONAL:**

1.3. **Erica J. Fratz-Berilla**: Since acoustic wave separation provides a continuous flow of cell-free material for subsequent filtration and chromatography processes, there is potential for its application in continuous bioprocessing **[1]**.

1.3.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera

# Protocol

## 2. Acoustic Wave Separator (AWS) Preparation

2.1. To prepare the acoustic wave separator, connect the turbidity cables to their respective ports **[1]** and connect the chamber power BNC (B-N-C) cables to the back of the acoustic wave separator system **[2-TXT]**.

2.1.1. WIDE: Talent connecting cable(s) *Videographer: Important step*

2.1.2. BNC cables being connected *Videographer: Important step* **TEXT: Do not connect cable to chamber until after fluid filling**

2.2. Insert the turbidity probes into the turbidity meter **[1]** and the thermometer housing **[2]** and tighten the screws **[3]**.

2.2.1. Probes being inserted into meter NOTE: 2.2.1 and 2.2.2 are combined

2.2.2. Probes being inserted into housing

2.2.3. Screw(s) being tightened

2.3. Connect the feed tubing to the input of the feed turbidity port via the feed pump **[1]** and connect the y-tubing from the output of the feed turbidity port to the inlet ports of the acoustophoretic chamber **[2]**.

2.3.1. Talent connecting tubing to turbidity port via feed pump
2.3.2. Talent connecting y-tubing to inlet port(s)

2.4. Connect the stage1 tubing from the waste port of the acoustophoretic chamber to a cell collection vessel via the stage1 pump **[1]** and connect the tubing from the permeate port of the acoustophoretic chamber to the input of probe1 turbidity port **[2]**.

2.4.1. Talent connecting stage1 tubing to stage1 pump
2.4.2. Talent connecting permeate port tubing to probe1 turbidity port

2.5. Then connect the harvest tubing from out of the probe1 turbidity port to a product collection vessel **[1]**.

2.5.1. Talent connecting harvest tubing to collection vessel

## 3. System Priming

3.1. To prime the system with harvested cell culture fluid, turn on the acoustic wave separator **[1]** and open the associated software program **[2]**.

3.1.1. WIDE: Talent turning on AWS NOTE: 3.1.1 and 3.1.2 are combined
3.1.2. Talent at computer, opening program, with monitor visible in frame

3.2. In the **Readings** panel, click **Start Test** to initiate the data recording **[1]** and connect the feed tubing end into the harvested cell culture fluid vessel being stirred **[2]**.

3.2.1. SCREEN: 2-2.mov.
3.2.2. Talent connecting tubing to vessel

3.3. To start the feed pump, enter the pump rate, and press **Enter** **[1]**.

3.3.1. SCREEN: 2-4.mov. 0:00 – 0:03.

3.4. Confirm that the **Pump Direction Arrow** is correctly oriented to ensure that the cell fluid is pumped from the vessel into the acoustophoretic chamber and click the **Triangle** icon to start **[1-TXT]**.

3.4.1. SCREEN: 2-4.mov. 0:04 – end. **TEXT: 60 mL/min recommended pump flow rate**

3.5. Monitor the feed turbidity measurements in the **Percent Reduction Panel** during the filling of the acoustophoretic chamber. If the harvested cell culture fluid is being mixed sufficiently within the cell fluid vessel, the turbidity values will be consistent during the loading of the chamber **[1]**.

3.5.1. SCREEN: Shot of Measurements *Videographer: Film the screen here. Video Editor: please emphasize turbidity values when mentioned*

3.6. Once the liquid is above the piezo transducer at the back of the acoustophoretic chamber **[1]**, click **Turn Off** to stop the pump **[2]** and connect the other end of the BNC power cable to the acoustophoretic chamber **[3]**.

3.6.1. Shot of liquid above transducer *Videographer: Important step*
3.6.2. SCREEN: Turn Off being clicked *Videographer: Film the screen here.*
3.6.3. Talent connecting cable to chamber

## 4. AWS Operation

4.1. Once the acoustophoretic chamber is filled and powered on, change the feed pump rate to the desired operating rate **[1]**.

4.1.1. WIDE: Talent change feed pump flow rate

4.2. To turn on the stage1 piezo power, slide the bar in the power module to 10 Watts and click **Turn ON** **[1]**.

4.2.1. SCREEN: Power being turned on, then bar being slid *Videographer: Film the screen here.*

4.3. Once the cells begin to settle to the bottom of the acoustophoretic chamber **[1]**, start the stage1 pump with an appropriate rate based on the cell density and feed pump rate **[2-TXT]** while watching the acoustic wave separator chambers closely **[3]** and adjusting the pump rates as necessary **[4]**.

4.3.1. Shot of cells settled at bottom of chamber *Videographer: Important step*
4.3.2. Talent starting pump *Videographer: Important step* **TEXT: Stage1 pump rate = feed pump rate × [2 × feed packed cell mass %]**
4.3.3. Shot of AWS chambers *Videographer: Important step*
4.3.4. Talent adjusting pump rate(s) *Videographer: Important step*

4.4. To calculate the packed cell mass, tare a scale with an empty 15-milliliter tube **[1]** and fill the tube with feed material **[2]**.

4.4.1. Talent placing empty tube onto balance
4.4.2. Talent adding feed material to tube, with feed material visible in frame

4.5. After recording the total weight of the tube with feed, centrifuge the tube **[1-TXT]** and decant the supernatant into a new container **[2]**.

4.5.1. Talent placing tube into centrifuge **TEXT: 10 min, 3700 x *g*, RT**
4.5.2. Supernatant being decanted

4.6. Measure the weight of the tube with the cell pellet **[1-TXT]**.

4.6.1. Talent placing tube onto balance **TEXT: Packed cell mass feed material percentage = (decanted tube weight/filled tube weight) x 100%**

4.7. Then monitor the turbidity profile of the stage1 turbidity **[1]** as the overflow from the acoustophoretic chamber enters the turbidity probe1 **[2]**.

4.7.1. Talent monitoring turbidity
4.7.2. LAB MEDIA: Figure 8

**5. Ending AWS**

5.1. When the run is over, stop the feed and stage1 pumps **[1]** and turn off the power to the chamber **[2]**.

5.1.1. WIDE: Talent turning off pumps, with monitor visible in frame NOTE: 5.1.1 and 5.1.2 are combined
5.1.2. Talent turning off power

5.2. Disconnect the BNC power cable **[1]** and collect the product harvest material for downstream analysis **[2-TXT]**.

5.2.1. Talent disconnecting cable(s)
5.2.2. Talent collecting material **TEXT: Discard cell harvest material**

5.3. To drain the remaining fluid from the acoustophoretic chamber, place the waste tubing into an empty vessel **[1]**, disconnect the tubing from the acoustophoretic chamber inlet and permeate ports **[2]**, and release the waste tubing from the pump head **[3]**.

5.3.1. Talent placing tubing into vessel
5.3.2. Talent disconnecting tubing
5.3.3. Talent releasing waste tubing

5.4. When the chamber is empty, reconnect the tubing **[1]**, place the waste tubing back into the pump head **[2]**, and use a feed and stage1 pump rate of 60 milliliters/minute to flush deionized water from the end of feed tubing for 15-20 minutes **[3-TXT]**.

5.4.1. Talent reconnecting tubing
5.4.2. Talent placing waste tubing into pump head
5.4.3. Water being flushed **TEXT: Discard flow through**

5.5. At the end of the water rinse, pump 70% isopropyl alcohol through the tubing and chamber for 15-20 minutes **[1]** followed by an additional 15-20-minute deionized water rinse **[2]**.

5.5.1. Talent adding IPA to tube(s), with IPA container visible in frame
5.5.2. Use 5.4.3. water being flushed

# Results

**6. Results: Representative Turbidity Measurements and Cell Removal Efficiency Comparison**

6.1. In this representative analysis, as the harvested cell fluid entered the acoustic wave separator chamber **[1]**, the turbidity measurements from the feed turbidity probe remained consistent, around 1000-1100 NTU (N-T-U) **[2-TXT]**, and measurements from the probe1 turbidity probe remained around 40-50 NTU **[3]**.

6.1.1. LAB MEDIA: Figure 8
6.1.2. LAB MEDIA: Figure 8 *Video Editor: please emphasize blue data line* **TEXT: NTU: nephelometric turbidity unit**
6.1.3. LAB MEDIA: Figure 8 *Video Editor: please emphasize orange data line*

6.2. Keeping the harvested cell fluid within the chamber for longer time periods causes temperature increases **[1]**, as in this example of a greater than 6-degree Celsius temperature difference of the harvested cell fluid before entering the acoustophoretic chamber **[2]** and after acoustic separation **[3]**.

6.2.1. LAB MEDIA: Figure 9
6.2.2. LAB MEDIA: Figure 9 *Video Editor: please emphasize blue data line*
6.2.3. LAB MEDIA: Figure 9 *Video Editor: please emphasize pink data line*

6.3. Another important consideration when running high cell density harvests is the saturation of the turbidity probes **[1]**, as the turbidity measurements for the feed turbidity probe become saturated over 4400 NTU **[2]**, likely resulting in an underestimation in the calculation of the cell removal efficiency **[3]**.

6.3.1. LAB MEDIA: Figure 10
6.3.2. LAB MEDIA: Figure 10 *Video Editor: please emphasize data line about 4400 NTU*
6.3.3. LAB MEDIA: Figure 10

6.4. In addition, the cell removal efficiency decreases significantly from approximately 100% to 57% as the feed pump rate is increased **[1]**, with a slower the feed pump rate typically directly correlating with a better cell clarification **[2]**.

6.4.1. LAB MEDIA: Figure 11 *Video Editor: please emphasize 1.5 and 3.5 ml/min data bars*
6.4.2. LAB MEDIA: Figure 11 *Video Editor: please emphasize 35 ml/min data bar*
6.4.3. LAB MEDIA: Figure 1 *Video Editor: please emphasize 1.5 and 3.5. ml data bars*

# Conclusion

**7. Conclusion Interview Statements**

7.1. **Erica J. Fratz-Berilla**: As manufacturers of biotechnology products move toward continuous manufacturing, the use of acoustic wave technology may have additional applications for continuous bioprocessing and perfusion bioreactor operations **[1]**.

7.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera