

Jung Example 3	'598 Patent Example	Inventor Declaration ('598 Patent PH)
“<i>Clostridium botulinum</i> was cultured using the medium 5 of Example 1 or 2.” EX1002 at 11:28-29. “The culturing conditions and time and the cell removal process were the same as those in Example 1.” <i>Id.</i> at 11:29-32. Medium 5: peptone 1%; <i>yeast extract</i> 1%; <i>glucose</i> 0.5%. <i>Id.</i> at Table 1. In Example 1, “200 ml of each of the media of Table 1 and the control media was filtered through a sterilized single use microfiltration system ... so as to be injected into Cultibag™ ... <i>Then, each media was inoculated with 1ml of a seed inoculum including Clostridium botulinum type A with a concentration of 10⁶-10⁷ cells/ml through a sample injection port.</i> Cultibag™ is a disposable bioreactor that may use rocking motion in order to cause mixing.” <i>Id.</i> at 8:56 – 9:2.	“In this Example, a large amount of <i>Clostridium botulinum</i> type A was expressed outside cells by <i>culturing Clostridium botulinum</i> in an animal product-free medium.” EX1001 at 7:23-25. “The used medium was a medium containing 4% <i>yeast extract</i> and 0.5% <i>glucose.</i>” <i>Id.</i> at 7:26-27. “200 ml of the medium thus prepared was filtered through a sterile single use microfiltration system ... and injected into Cellbag™ ... <i>Then, the medium was inoculated with 1 ml of a seed culture of Clostridium botulinum at a density of 10⁶ cells/ml – 10⁷ cells/ml through a sample injection port.</i> Cellbag™ is a disposable bioreactor that uses rocking motion in order to cause mixing under low shear stress.” <i>Id.</i> at 7:31-39.	“In this experiment, a large amount of <i>Clostridium botulinum</i> type A toxin was expressed outside cells by <i>culturing Clostridium botulinum</i> in an animal product-free medium.” EX1006, 7-22-2021 Declaration at 1. “The used medium was a medium containing 4% <i>yeast extract</i> and 0.5% <i>glucose.</i>” <i>Id.</i> “200 ml of the medium thus prepared was filtered through a sterile single use microfiltration system ... and injected into Cellbag™ ... <i>Then, the medium was inoculated with 1 ml of a seed culture of Clostridium botulinum at a density of 10⁶ cells/ml-10⁷ cells/ml through a sample injection port.</i> Cellbag™ is a disposable bioreactor that uses rocking motion in order to cause mixing under low shear stress.” <i>Id.</i> at 2.

“After the culturing was terminated, a sterile connector QDC connector (manufactured by Satorious) was connected to the sample port of the bag ... and the culture was filtered through depthfilter and the microfilter to remove the cell.” <i>Id.</i> at 9:36-46.	“After the culturing was terminated, a QDC connector (manufactured by Satorious) which is a sterile connector was connected to the sample port of the bag... and the culture was filtered therethrough to remove the cells.” <i>Id.</i> at 8:1-9.	“After the culturing was terminated, a sterile connector QDC connector (manufactured by Satorious) which is a sterile connector was connected to the sample port of the bag ... and the culture was filtered therethrough to remove the cells.” <i>Id.</i>
“0.1 N sulfuric acid was added to the bag including 20 L of a culture supernatant to adjust the pH of the resulting solution to be in the range of 3.0 to 4.5, and as a result, toxin protein was precipitated.” <i>Id.</i> at 11:33-36.	“1 N sulfuric acid was added to a bag containing 20 L of the culture supernatant obtained as above to adjust pH from 3.0 to 4.5, and as a result, toxin protein was precipitated.” <i>Id.</i> at 8:19-21.	“1 N sulfuric acid was added to a bag containing 20 L of the culture supernatant obtained as above to adjust pH from 3.0 to 4.5, and as a result, toxin protein was precipitated.” <i>Id.</i> at 3.
“10 L of a 25 mM citrate buffer (pH 5.5) was added to the collected precipitate to re-dissolve toxin protein existing in the precipitation.” <i>Id.</i> at 11:51-53.	“10 L of citrate buffer (25 mM, pH 5.5) was added to the collected precipitate to redissolve toxin protein existing in the precipitate.” <i>Id.</i> at 37-39.	“10 L of citrate buffer (25 mM, pH 5.5) was added to the collected precipitate to redissolve toxin protein existing in the precipitate.” <i>Id.</i>
“[F]iltration was performed on the toxin protein through the system not in contact with the outside. As a result, the supernatant was collected in a sterilized disposable bag and insoluble impurities were removed... and a buffer exchange	“[F]iltration was performed without being in contact with the outside. As a result, the supernatant was collected in a sterile disposable bag and insoluble impurities were removed.” <i>Id.</i> at 8:43-46.	“[F]iltration was performed without being in contact with the outside. As a result, the supernatant was collected in a sterile disposable bag and insoluble impurities were removed.” <i>Id.</i>

was performed on the enriched resultant by using a 25 mM citrate buffer (pH 5.5).” <i>Id.</i> at 11:57 – 12:1.	“Then, DEAE anion exchange chromatography and membrane chromatography were performed ... After the sample was loaded on the system, the <i>Clostridium botulinum type A toxin protein in the sample was not bound to a column</i> while other impurities [<i>sic</i>] were bound to the column, and <i>thus a fraction flowing out of the system was collected.</i>” <i>Id.</i> at 12:4-16.	“DEAE anion exchange chromatography was performed ... In detail, ... the toxin-containing <i>citrate buffer (25 mM pH 5.5)</i> was passed through the column. In this process, <i>Clostridium botulinum type A toxin was not adsorbed to the anion exchange resin</i>, and most impurities were adsorbed thereto and removed.” <i>Id.</i> at 8:60 – 9:2. “Next, <i>a fraction containing Clostridium botulinum type A toxin was collected from the flow-through solutions</i> which were not bound to the anion exchange resin during the anion exchange chromatography.” <i>Id.</i> at 9:5-8.	“Q anion exchange chromatography was performed [and] [t]he flow through solution was analyzed.” <i>Id.</i> “As shown in FIG. 1, the botulinum toxin containing sample was detected immediately after loading the sample ... This indicates that the <i>botulinum toxin is contained in the flow through solution.</i>” <i>Id.</i>
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