

CLAIMS

1. A method of detecting an endotoxin in a sample using a chromogenic assay, the method comprising:
 - a) contacting the sample with a reagent comprising limulus amebocyte lysate (LAL) and a chromogenic substrate;
 - b) measuring a chromogenic effect resulting from a change in the chromogenic substrate in the presence of endotoxin in the sample;wherein the LAL has been (i) filtered using tangential flow filtration with a 20 kDa to 50kDa filter configured to remove a portion of its coagulogen, and then (ii) subjected to centrifugation, and
wherein the method is capable of detecting endotoxin at a concentration of 0.0005 EU/mL,
wherein the filter is selected from the group consisting of: polyethersulfone (mPES), polysulfone (PS) and polyethersulfone (PES).
2. The method of claim 1, wherein the chromogenic substrate is Ac-Ile-Glu-Ala-Arg-pNA.
3. The method of claim 1, wherein the sample is a biological sample.
4. The method of claim 1, wherein the sample is selected from the group consisting of a parenteral dosage form, vaccine, antibiotic, therapeutic protein, therapeutic nucleic acid, therapeutic antibody, and biological product.
5. A method of making clarified limulus amebocyte lysate (LAL) substantially free of coagulogen, the method comprising:
 - a. centrifuging a solution derived from lysed amebocytes from *Limulus polyphemus* at 1000 to 3000 rpm for 2 to 15 minutes at 2 to 10°C to produce a supernatant;
 - b. combining the supernatant from (a) with a buffer;
 - c. filtering the combination from (b) using tangential flow filtration with a 20 kDa to 50 kDa filter to produce a retentate, wherein the filter is selected from the group consisting of: polyethersulfone (mPES), polysulfone (PS) and polyethersulfone (PES);

- d. centrifuging the retentate from (c) at 3000 to 7000 rpm for 2 to 10 minutes at 2 to 10°C to produce a supernatant, wherein the supernatant comprises clarified LAL that is substantially free of coagulogen.
6. The method of claim 5, wherein the filtering of (c) comprises using a 30kDa filter.
7. A method of making clarified limulus amebocyte lysate (LAL) substantially free of coagulogen, the method comprising:
 - a. obtaining a solution derived from lysed amebocytes from *Limulus polyphemus*;
 - b. combining the solution from (a) with a buffer;
 - c. subjecting the combination from (b) to continuous tangential flow filtration (TFF) using a 20 kDa to 50 kDa membrane filter to produce a retentate, wherein the filter is selected from the group consisting of: polyethersulfone (mPES), polysulfone (PS) and polyethersulfone (PES); and
 - d. centrifuging the retentate from (c) at greater than 20,000 x g to produce a supernatant, wherein the supernatant is clarified LAL that is substantially free of coagulogen.
8. The method of claim 1, wherein the LAL has less than 30% (wt/wt) of coagulogen relative to total protein in the LAL as measured by SDS-PAGE and protein stain.
9. The method of claim 1, wherein the LAL is visibly clear.
10. The method of claim 1, wherein the LAL has an optical density absorbance of about 1 to about 1.1 at 300 nm, about 0.2 to about 0.4 at 350 nm, about 0.1 to about 0.2 at 400 nm, about 0.1 to about 0.15 at 450 nm, about 0.1 at 500 nm, or combination thereof.
11. The method of claim 1, wherein the method is capable of detecting endotoxin at a concentration of 0.0005 EU/mL in a kinetic assay.