Fish Lens Cataracts Model

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Materials:

- Tris buffer
  - Trizma® base (Primary Standard and Buffer, ≥99.9% (titration), crystalline)
  - DL-Dithiothreitol (≥98% (TLC), ≥99.0% (titration))
  - EDGMA Ethylenediaminetetraacetic acid
- Priacanthus macracanthus (from fish market)
- 25-hydroxycholesterol (≥98%)
- L-Glutathione oxidized (≥98% (HPLC))
- L-Glutathione reduced (≥98.0%)

Procedure:

- **Pre-lab: Find an appropriate wavelength to measure absorbances**
  - Prepare 2 groups of fish nuclear protein (in dh2o)
    - Control (no treatment)
    - + 200uL of 35% h2o2
  - Let sit for 10 min
  - Measure Abs with SpectroVis
    - Blank with water
    - Get full spectrum
  - Results: Highest absorbance at 397.5 nm

- **Day 1:**
  - Dissect fish (紅目鰱) eyes, extract both lenses (nucleus)
    - Rinse/clean gently with 2 mL Tris buffer
  - Place each sample in 12~16 mL Tris buffer (15mL centrifuge tube)
  - Tape onto benchtop rotator and gently shake overnight at 70rpm
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Hydrogel Contact Lenses

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Materials:

- Hydroxyethylmethacrylate (HEMA (≥99%, contains ≤50 ppm monomethyl ether hydroquinine as inhibitor) (Sigma-Aldrich)
- Ethylene glycol dimethacrylate (EGDMA) (98%, containing 90–110 ppm monomethyl ether hydroquinone as inhibitor) (Sigma Aldrich)
- 2,4,6-Trimethylbenzoyl-diphenyl-phosphineoxide (TPO)

Procedure:

1. Mix 2.7 ml of HEMA with 2 ml of DI water and 10 µl of EGDMA in a clean glass vial.
    a. If nanoparticles, add now
2. Bubble solution with nitrogen for 15 mins (to deoxygenate it)
    a. Seal cap immediately after bubbling
3. Once bubbling process is finished, add 6mg TPO to solution and mix for 5 mins at 800 rpm
4. Somehow make a mould, and pour the solution into the mould after mixing
5. Place filled mould on UV machine for 40 mins for photo polymerization

Chitosan Nanoparticle (w/ Protein)

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Materials

Sigma-Aldrich Chitosan (Low Molecular weight)
Choneye Sodium Tripolyphosphate
Glacial Acetic Acid
Procedure:

1. **Making Stock Chitosan and TPP aqueous solution**

Prepare two set of distilled water of equal volume in 15ml centrifuge tube. Label one tube as "Chitosan" and the other as TPP. (*50ml tube for stock solution is not recommended to have the solution be less exposed to moisture*.)

Add 1% by volume glacial acetic acid into the Chitosan tube. Mix vigorously.

Add 3mg/ml of Chitosan to the Chitosan tube and 1mg/ml of TPP to the TPP tube.

Mix both tubes vigorously to each dissolve Chitosan and TPP.

Adjust the pH of the Chitosan solution with 1M NaOH to pH 5.5

2. **Mixing Chitosan and TPP solutions.**

Pour the desired amount of Chitosan Solution in a beaker.

Add desired amount of protein into the Chitosan Tube. Dissolve by gently swirling.

Add equal amount of TPP solution as chitosan solution but dropwise into the beaker under 800rpm of magnetic stirring.

The solution should turn turbid after the addition of all the TPP solution.

3. **Centrifuging the Chitosan Nanoparticle**

Centrifuge the nanoparticle suspension at 17000 xg for 40 minutes at 4 degrees Celsius.

Remove the supernatant to obtain a pellet of nanoparticles with encapsulated protein

Protein Purification

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This protocol is based off of **Clontech Capturem Histidine-tag Miniprep Purification kit Protocol**

Material (provided by Clontech Miniprep kit)

xTractor Buffer
Wash Buffer
Elution Buffer
ProteoGuard™ EDTA-Free Protease Inhibitor Cocktail (Dissolved in water to make 100X concentration)

Procedure:

1. **Harvest cell culture**

Harvest the bacterial cell culture by centrifugation at 1,000–3,000 x g for 15 min at 4°C. (The liquid culture depends on the amount of desired protein need be purified)

Remove the supernatant.

Store cell pellet at –20°C for long-term storage or proceed to the next step.

2. **Resuspend the cell pellet**

Add 20 ml of xTractor Buffer to 1 g of cell pellet.

NOTE: A log-phase E. coli culture (O.D.=0.6–0.8), when induced for 2–4 hours, would be expected to provide ~20–40 mg of bacterial pellet from 3 ml of the culture. Assume 40mg of pellet. Add 800uL of xTractor Buffer.

Mix gently. Pipet the mixture up and down to fully resuspend pellet

3. **Add in 1x Protease inhibitor Cocktail**

Add 1x EDTAfree protease inhibitor. Swirl Gently. Do not pipette or vortex.

4.**Incubation**

Incubate with gentle shaking for 10 min at room temperature.

NOTES: At the end of the incubation period, there should be **no visible particles**. If cell pellet fragments are present, resuspend them by pipetting the solution up and down and incubating for an additional 1–2 min.

5. **Lysate Clarification**

Centrifuge the crude lysate at 10,000–12,000 x g for 20 min at 4°C. Carefully transfer the supernatant (clear lysate) to a clean tube without disturbing the pellet. Store in ice or at 4 degrees fridge or proceed

to next step.

6. **Equilibrate the Nickel Resin Column**

   Add 400uL xTractor Buffer to a spin column which has been placed in the collection tube (equilibrate the column). Centrifuge at 11,000 xg for 1 minute at room temperature. Remove flow through AND column, then place a new collection tube under the original column.

7. **Binding His-tagged proteins to the column**
   Load 200-800uL of lysate (from Protocol part 1) in the spin column. Centrifuge at 11,000 x g for 1 minute at room temperature. SAVE THE COLLECTION TUBE AND TRANSFER FLOW THROUGH CONTENTS TO AN EPPENDORF. Get a new collection tube and put it under the same column. Repeat with new collection tubes until all clear lysate is spun down.

   Washing the column
8. Add 300uL Wash Buffer to the spin column. Centrifuge at 11,000 xg for 1 minute at room temperature. SAVE THE COLLECTION TUBE AND TRANSFER FLOW THROUGH CONTENTS TO AN EPPENDORF. Get a new collection tube and put it under the same column.

   Eluting the his-tagged proteins.
9. Add 300uL Elution Buffer to the spin column. Centrifuge at 11,000 xg for 1 minute at room temperature. SAVE THE COLLECTION TUBE AND TRANSFER FLOW THROUGH CONTENTS TO AN EPPENDORF. (If using GFP, this tube should be the "greenest" since most of the proteins should be here)

   Protein Analysis
10. Use Nanodrop or carry out Bradford assay to find the his-tagged protein concentration. Run protein gel to check if the right band protein is collected.

Protein Release

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Made nanoparticles with 7 mL TPP solution and 7 mL chitosan solution with 14 mg BSA
Made nanoparticles with 4 mL TPP solution and 4 mL chitosan solution without proteins as blanks
transferred 1 mL of nanoparticle suspension into an microcentrifuge tube and centrifuged at 17000xg
Removed the supernatant and added 1 mL PBS into each tube
Resuspended the nanoparticles
Made standard BSA solutions by adding certain amounts of BSA to the blank samples
We performed Bradford assay on the standard solutions to obtain a standard curve
We measured the initial absorbances
We incubated 4 samples at 37C and the other 4 at 4C
We used Bradford Assay to measure protein release at 24, 48, and 72 hours
For measurement, we first centrifuged the nanoparticles suspension
We took 40 uL of the supernatant (40 uL of PBS as blank) and added 2 mL Coomassie Brilliant Blue
After 5 minutes, we measured the absorbance at 595nm

Protein Encapsulation efficiency

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To make nanoparticles containing BSA, we dissolved 2 mg/mL of BSA in the chitosan solution and added an equal volume of TPP solution.
We also made empty nanoparticles without adding any BSA.
We centrifuged the samples and measured the protein concentration of the supernatants to find the encapsulation efficiency.

We took 40uL of the supernatant and added 2 mL of Coomassie Brilliant Blue (Bio-rad) and measured the absorbance at 595nm.
We converted the absorbance value to concentration using a standard curve

To make the standard solutions, we dissolved measured amounts of BSA in the supernatant of the blank samples.
We made standard solutions of concentrations 0.25, 0.5, 0.75, 1.0, 1.5, 2.0 mg/mL of BSA.
We took 40uL of the standard solutions and added 2 mL of Coomassie Brilliant Blue (Bio-rad) and measured the absorbance at 595nm.
We fitted the absorbances to get a standard curve.

To find the encapsulation efficiency, we used this formula:
Encapsulation efficiency = (1 - protein concentration in supernatant)/1 * 100%

Since we dissolved 2 mg/mL BSA in chitosan solution and added an equal volume of TPP, we expected the protein concentration in the supernatant to be 1 mg/mL if proteins were not encapsulated. Therefore the amount of protein encapsulated in the nanoparticles is the protein concentration in the supernatant subtracted from 1 mg/mL. We multiply that value by 100% to get the percentage.

GFP RFP pGRN encapsulation

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Lysis

We cultured 30 mL of GFP RFP and pGRN overnight
We centrifuged and decanted the supernatant
Added 100 uL of lysis buffer to each pellet and mixed using pipet and vortex
We centrifuged the crude lysates and extracted the supernatant to obtain the protein containing lysates

Nanoparticles

We added 80 uL of each lysate into 0.5 mL of chitosan solution
Made and centrifuged the nanoparticles
We extracted the pellet and patted the nanoparticles dry
We observed the nanoparticles under blue light