Preparation of fish serum for the culture of marine fish cell lines

1. Fish is anesthetized with 2-methoxy-4-propenylphenol diluted 1:5000 in seawater. After approx. 2-5 min fish become sluggish and can be handled easily.
2. Cover fish head with a damp cloth to prevent movements from muscle twitching.
3. Maintain the fish in a dorsal position and insert the needle next to the anal fin towards the vertebral column. Partially pull out the plunger of the syringe to create a small vacuum so that blood flows into the syringe when vein is punctured.

   Alternative: Maintain the fish in a lateral position Insert the needle below the lateral line (approx. half way between the ventral and dorsal surface) and at 2-3 cm from the caudal fin.
4. Pull out syringe plunger until sufficient blood has been collected then remove the needle.

   Approximately 5 ml of blood is collected from a gilthead seabream with a body weight of about 500 g (~ 1%).
5. Place the fish in a container with fresh seawater and monitor its recovery.
6. Transfer blood into glass tubes and allow it to clot at room temperature for 2 h.
7. Contract blood clot for 4 h at 4°C then separate clot from serum by centrifugation (1590 rcf for 10 min at 4°C).
8. Carefully transfer the serum (i.e. supernatant fraction) into a new tube.

   Serum fraction represents approximately 30-40% of total blood fraction.
9. Pool the serum collected from various fish and filter-sterilize using 0.2 µm filters; Store aliquots (5 mL) at -80°C.

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Fish: Healthy adults of marine fish bred in aquaculture conditions (body weight of approx. 500-700 g.)

Apparatus: Centrifuge.

Solutions: 2-methoxy-4-propenylphenol (Aqui-S New Zealand Limited Ltd)

Material: 1.5 mL syringes, 23 gauge 1'' needles, glass tubes, 5 mL polypropylene tubes, ice bath.

All chemicals were purchased from Sigma-Aldrich, unless otherwise stated.

Additional information:
***Rosa J, Tiago DM, Dias J, Cancela ML, Laizé V** (2010) Serum-specific stimulation of proliferation and mineralization of fish bone-derived cells. J Appl Ichthyol 26:251-256*

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