

Jimenez lab: Dog aging

Tissue Culture Procedure:

Transfer Media make-up: (I will provide this to you in 15 mL conical tubes, as shown to the right, that are to be kept in the refrigerator at all times. Skin viability decreases quickly after it is isolated from the animal and temperature fluctuations expedite this process exponentially)



Into 500 mL of DMEM (High Glucose/Pyruvate/Glutamine) add:

- 1) 50 mL of FBS*
- 2) 5 mL fungizone
- 3) 5 mL Pen/Strep antibiotic (10,000 mg/ mL)
- 4) 10 mM HEPES tissue culture grade
- 5) This mixture is further sterilized by filtering it through 0.22 micron pour size to eliminate all bacteria.

Once you receive our box, please place the transfer media tubes in your fridge right-side up until you are ready to collect samples, and move the ice packs to your freezer until you are ready to mail the samples back to us.

Tail docks/earclips/dewclaws:

At your veterinarian's office, please place the removed tail-dock/ear clip/dewclaw in transfer media tube so that the tissue samples are fully submerged in media at all times. Close lids tightly and re-wrap with parafilm (plastic wrap), so as to prevent spillage on transit.

If you are removing and sending us both dewclaws and tail docks, please place both of these tissues in a single vial per puppy.

Each tube should be labelled with:

- Dog's name (or ID)
- Dog's breed (if mixed, which breeds?)
- Dog's weight or average weight of the litter
- Dog's age
- Dog's gender

Move transport media to **fridge as soon as the skin has been placed in the tube.** Always keep skin samples cold. Once you have collected samples, please let us know so we can provide you with a FedEx or UPS label to ship back to us. Please, give us a heads up when samples are headed our way!

PLEASE NOTE: If you have more puppies than vials, please, DO NOT combine tails of different individuals into a single vial.

IMPORTANT: Skin is only viable for up to ~72 hours in transfer media, so it is of utmost importance that samples are mailed as soon as they are isolated from the dog.

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