

overt diceCT protocol

Lab protocol by Dr. Jaimi A. Gray

Step 1

STANDARD CT SCAN

Step 2

WALK DOWN ETOH CONCENTRATION 2-3 days for each step down



70%

Museum specimens are stored in 70% EtOH



50%

To make 50% EtOH
Add 40 ml H₂O for every 100 ml 70% EtOH



30%

To make 30% EtOH
Add 130 ml H₂O for every 100 ml 70% EtOH

Step 3

STAIN WITH BUFFERED LUGOL'S IODINE SOLUTION

Prepare a stock solution of 15% Lugol's iodine

5 g of I₂ and 10 g of KI, made up to 100 ml de-ionized H₂O.

For storage jugs in herp lab...

185 g I₂
380 g KI
3.8 L total

2.65 L Na₂HPO₄
1.05 L KH₂PO₄
3.8 L total

Prepare a two times Sorensen's buffer

For 266 mM Na₂HPO₄
Mix 37.76 g Na₂HPO₄ in 1 L de-ionized H₂O
For 266 mM KH₂PO₄
Mi 36.20 g KH₂PO₄ in 1 L de-ionized H₂O

Test for pH = 7.2

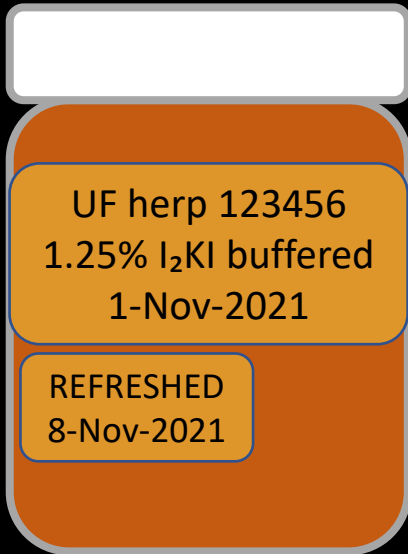
Prepare buffered Lugol's solution of target concentration

Target %	Volume of 15% Lugol's	Volume of DI H ₂ O	Volume of buffer	Total volume
1.25	8.33	41.67	50	100
2.50	16.67	33.33	50	100
3.75	25	25	50	100

Submerge specimen in prepared solution

1.25% I₂KI

for most specimens



3.75% I₂KI

for large or difficult specimens

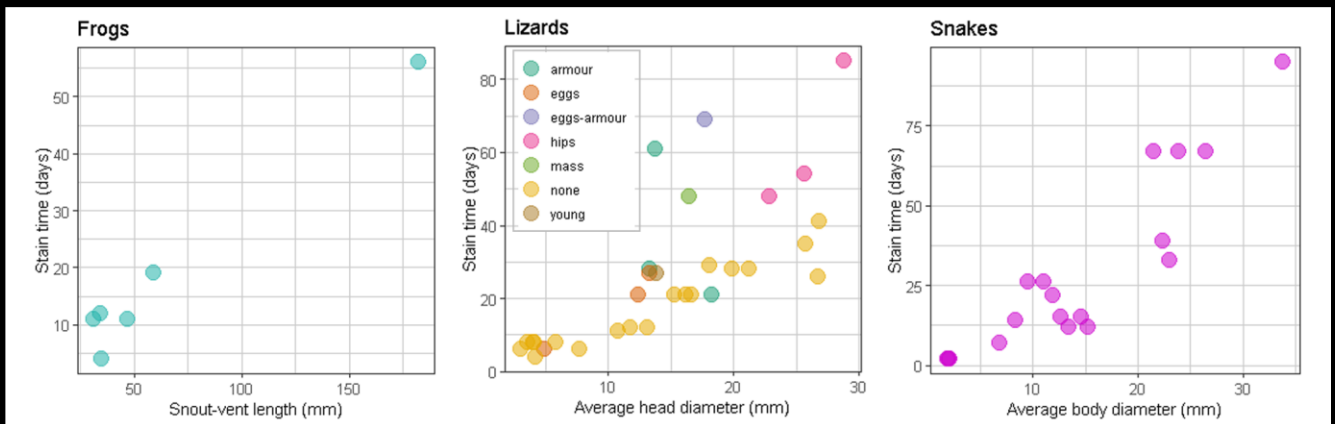
Choose a staining vessel with plenty of room for enough staining solution. If possible, vessel should seem “too big” for specimen.

Label each staining vessel with

- Specimen number
- Staining solution
- Date in stain
- New label with date for each solution refresh

Keep an eye on solution as staining progresses and refresh solution if it starts to look pale.

Staining times for 1.25% I₂KI, and body measurements for frogs (SVL), lizards (head diameter), and snakes (body diameter) of different sizes.



Stain time can be predicted using body/head size measurements, and the best predictor of stain time will depend on body shape. Some other factors, such as armour, presence of eggs, or presence of large hip muscles, will affect staining times.

Assess stain progression

When specimen has been in solution for sufficient time, conduct 5-10 min rapid scan to assess staining progression. If iodine diffusion gradient is visible (see image), return to staining vessel and refresh solution.



Step 4

DIFFERENTIATION

Removes unbound iodine and helps even out overstained surfaces
Submerge specimen in 30% EtOH bath before scanning

Amphibians
~ 30 minutes

Reptiles
~ 60 minutes

Specimens with
overstained extremities
> 1 hour

Specimen with excessive overstaining can be left in EtOH bath for up to 24 hours

Step 5

DIRECT SCANNING

Packing – try to pack specimen so that external surfaces are not touching, and use low density foam to separate parts of the body.

Scanning – increase voltage, current, and filtering for stained specimens.

CT scan stained specimen

Step 6

DESTAINING

Walk the EtOH concentration back up
2-3 days for each step up



Leave in 70% EtOH

Refresh the solution regularly until destaining is complete. Iodine will leach back out of specimen, and larger specimens will take longer to destain. Destaining is complete when EtOH remains completely clear.