Von Kossa staining is commonly used to detect the presence of calcium deposits in tissues. Here's a simple protocol you can use for your snap-frozen cardiac tissue:

Materials:

- %(?) silver nitrate solution

- Distilled water

- Sodium thiosulfate

- Nuclear fast red solution (optional)

- Glycerol-based mounting medium (if desired for long term preservation)

- Glass slides and coverslips

Procedure:

1. **Preparation of the tissue slides:** Begin by preparing thin sections of your snap-frozen cardiac tissue. Typically, 5-6 µm sections are used. Mount the sections onto glass slides. Allow the slides to air-dry.

2. **Fixation:** Fix the tissue slices in acetone at -20°C for 10 minutes or use a mixture of acetone and chloroform (3:1 ratio) at room temperature for 20 minutes. This will preserve the tissue morphology and enhance staining.

3. **Rinsing:** Rinse the slides in tap water for 3 minutes to remove the fixative.

4. **Silver nitrate incubation:** Incubate the slides in 5% silver nitrate solution under UV light for 30-60 minutes or until a brown/black precipitate forms. This step will help visualize the calcium deposits. The UV light reduces the silver ions to elemental silver.

* Slide 1/4: 20 mins
* Slide 2/4: 20 mins

5. **Rinsing:** Rinse the slides in tap water for 1 minutes to remove excess silver nitrate.

6. **Sodium thiosulfate incubation:** Incubate the slides in 5% sodium thiosulfate solution for 5 minutes to remove unreacted silver. Sodium thiosulfate helps to "fix" the silver staining by reacting with unbound silver ions.

7. **Counterstaining (optional):** Rinse the slides again in distilled water and then counterstain with nuclear fast red for 3 minutes if you wish to visualize the cell nuclei.

* Slide 1/4: 3 mins
* Slide 2/4: 3 mins

8. **Final Rinsing:** Rinse the slides in distilled water again.

9. **Mounting:** Air dry the slides or dehydrate in graded alcohols, clear in xylene, and mount in a resinous mounting medium. If the slides will be viewed soon after staining, you can instead use a glycerol-based mounting medium and coverslip.

* Graded dehydration à Xylene (2 mins) à Air dry à Dehydrate in 100% EtOH (20s) à Xylene (20s) à Mount

Please note that different protocols may call for slight variations in these steps, and it may be necessary to optimize the procedure for your specific samples. Always remember to include a known positive control and a negative control (the latter can be treated identically except without the silver nitrate) when performing any staining procedure.