Fluorescence *In situ* Hybridization (FISH) Protocol for Decalcification:

Protocol:

- Fixation of the bone marrow tissue should occur in 10% neutrally buffered formalin for at least 24 hours.
- For decalcification we recommend using an EDTA solution:
  200 g EDTA disodium salt(and approx. 20g NaOH bring to 1000 ml with distilled water (pH should be 7.0-7.2).
- Decalcify for 24 to 48 hours.
- Then wash in tap water for no longer than 30 min!
- Dehydrate and embed with paraffin:
  60 min 70% isopropanol(or ethanol)
  60 min 96% isopropanol(or ethanol)
  60 min 96% isopropanol(or ethanol)
  60 min 99% isopropanol(or ethanol)
  60 min 99% isopropanol(or ethanol)
  60 min 99% isopropanol(or ethanol)
  60 min xylene
  60 min xylene
  60 min paraffin
  60 min paraffin
- Proceed with standard FISH protocol.

Other decalcification methods will not be compatible with FISH analysis!