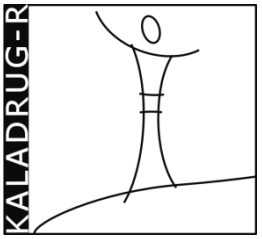
KALADRUG-R: Clinical SOP#B

Bone marrow/spleen aspirates for PCR & DNA extraction



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A. Material required

1. Screw cap tubes
2. EDTA (see preparation EDTA solution in point B, alternatively heparin can be used)

B. Preparation of EDTA - solution

EDTA : Ethylenediaminetetraacetic acid disodium salt dihydrate (Mol. biology grade)

1. Add 1.6 g EDTA to 1000 mL distilled water
2. Use a magnetic stirrer to dissolve the EDTA completely
3. Adjust pH=7.4 by adding HCl or NaOH
4. Sterilize by filtration using 0.22µm filter
5. Divide in aliquots of 10 ml and store at 4°C
6. Final Concentration 1.6 mg/ml: use 0.1ml stock solution/ml blood or bone marrow

C. Sampling and storage

1. Label screw cap tube as specified in cSOP#E/F
2. One mL of bone marrow (BM) or spleen (SP) for PCR should be mixed immediately after sampling in a screw cap tube containing 0.1 mL EDTA and stored at -80°C.
3. Register the clinical samples in the log book, clearly noting Kaladrug-R number and storage space.
4. Extract the DNA with the QIAamp DNA blood mini extraction kit (see point D. DNA extraction of BM/SP)
5. Register DNA extraction + used controls in the DNA extraction log book, clearly noting Kaladrug-R number and storage space of DNA vials.

D. QIAamp protocol (QIAamp DNA Mini Kit, Qiagen; cat.no: 51304) for DNA extraction of bone marrow or spleen samples

1. Equilibrate sample to room temperature.
2. If not done yet, transfer sample (in EDTA or heparin) in a 2 ml microcentrifuge tube and add 180 µl of buffer ATL.
3. Add 20 µl Proteinase K, mix by vortexing, and incubate at 56°C till tissue is completely lysed. Lysis times vary between 1-3 hrs (lysis overnight is possible).
4. Add 200 µl AL buffer, mix by vortex for 15s and incubate at 70°C for 10 min.
5. Add 200 µl ethanol (100%) and mix thoroughly (vortex 15 sec.)
6. Carefully apply the mixture from step 5 to the QIAamp Mini spin column, centrifuge at 6000g for 1 min. and place the QIAamp Mini spin column in a clean 2 ml collection tube and discard the filtrate.
7. Add 500 µl AW1 buffer and centrifuge 1 min (6000g); discard the filtrate.
8. Add 500 µl AW2 buffer and centrifuge 3 min at full speed; discard the filtrate.
9. Place the spin column in a clean 1.5 ml microcentrifuge tube, add 100-200 µL AE buffer, incubate for 1 min at room temperature.
10. Centrifuge 1 min (6000g)
11. Store at -20°C