

SAMPLE PROCESSING PROTOCOLS

for
Biliary Tract Cancers

SAMPLE PROCESSING PROTOCOLS

SERUM

- 1 Collect blood in serum tube
- 2 Keep upright 30 min at Room Temperature
- 3 Centrifuge at 1,500g, 20 minutes, at Room Temperature
- 4 Aliquote the serum in volumes of 1 ml
- 5 Freeze at -80°C

PLASMA

- 1 Collect blood in EDTA tube
- 2 Centrifuge at 1,500g, 20 minutes, at Room Temperature
- 3 Aliquote the plasma in volumes of 1 ml
- 3.1 Collect gDNA with Flexigen DNA Kit (Automated or Manually)
- 4 Freeze at -80°C

BILE

- 1 Collect bile
- 2 Centrifuge at 1,500g, 20 minutes, at Room Temperature
- 3 Aliquote the bile in volumes of 1 ml
- 4 Freeze at -80°C

URINE

- 1 Collect the urine
- 2 Aliquote 3 ml of urine in volumes of 1 ml
- 2.1 Centrifuge the remainder urine at 3,000g, 5 minutes, at Room Temperature
- 2.2 Aliquote the urine in volumes of 1 ml
- 3 Freeze at -80°C

STOOLS

- 1 Collect stools
- 2 Freeze at -80°C

SALIVA

- 1 Collect saliva with Orogen DNA kit (Genotek)
- 2 Follow the protocol

TUMOR TISSUE AND SURROUNDING TISSUE

Frozen

- 1 Cut a piece of non-tumoral and tumoral tissue
- 2 Put them in eppendorf tubes and store them at -80°C

Frozen on OCT

- 1 Cut a piece of non-tumoral and tumoral tissue
- 2 Put each piece in a cryomold with OCT
- 3 Immerse the cryomolds into isopentane at -80°C
- 4 Keep the cryomolds for 5-10 minutes in isopentane
- 5 Leave it drying and storage at -80°C
- 6 If there is enough tissue, put also a piece of non-tumoral and tumoral tissue in an eppendorf
- 7 Immerse the eppendorfs into isopentane at -80°C
- 8 Storage at -80°C

Embedded in paraffin

- 1 Cut a piece of non-tumoral and tumoral tissue
- 2 Put each piece in a cassette
- 3 Immerse the cassettes in formaldehyde 4%
- 4 Keep in the formaldehyde until the next day
- 5 Introduce the cassettes in Ethanol 96% during 1h 30 minutes (x2) and then introduce them in Ethanol 96% for 1h
- 6 Put the cassettes in Ethanol 100% for 1h 30 minutes (x2)
- 7 Place the cassettes in xilol for 1 h (x3)
- 8 Soak up the cassettes in paraffin at 55-60°C for 2 h (x2)
- 9 Put the paraffined cassettes on a “cold plate” to form the block 10- Cut and make the slides