

Preparations for tissue measurements in LigandTracer®

This protocol describes suitable preparations for the measurement of paraffin embedded tissue samples in LigandTracer.

Important information

Results may vary between tissues and lab. For example, that the paraffin embedding procedures and the storage of the samples may have an impact on tissue attachment and antigen activity.

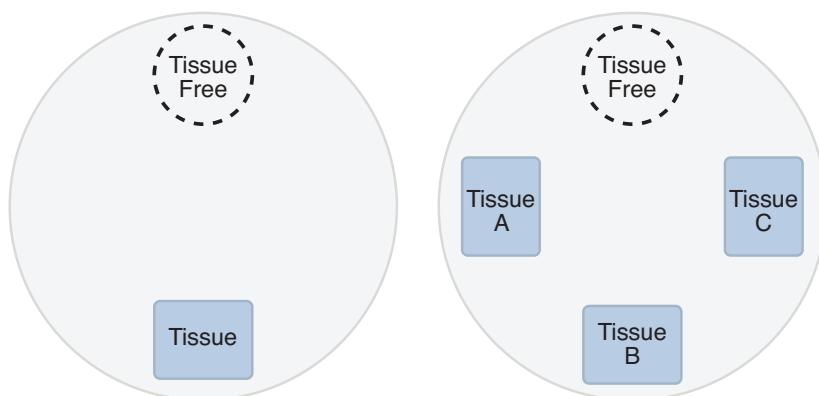
Materials

- Soda glass dish, 87-89 mm (diameter), about 15 mm (height)
- 2 % silane (3-aminopropyltriethoxysilane) in acetone
- 70 % ethanol
- 95 % ethanol
- Absolute ethanol
- Acetone
- Xylene
- 0.01 M citric buffer, pH 6
- De-ionized H₂O

Procedure

Prepare dishes

1. Clean dishes in 70 % ethanol.
2. Immerse dishes with 2 % silane in acetone for 10 s.
3. Wash dishes with acetone for 10 s.
4. Wash dishes with de-ionized H₂O for 10 s.
5. Air dry dishes over night or heat at 50°C for 1 h.
6. Cut 2-20 µm thick sections of tissue and add close to the rim of the dish. Up to three tissue sections may be added to one dish. Keep one area tissue free for reference subtraction.
7. Incubate in 55°C over night.



Paraffin removal

1. Immerse dishes with xylene for 20 min.
2. Wash in xylene for 5 min.
3. Immerse dishes with absolute ethanol for 20 min.
4. Wash in absolute ethanol for 5 min
5. Immerse dishes with 95 % ethanol for 20 min.
6. Wash in 95 % ethanol for 5 min
7. Immerse dishes with 70 % ethanol for 20 min.
8. Store in de-ionized H₂O.

Heat retrieval

1. Heat dishes in a beaker with 90°C 0.01 M citric buffer (pH 6) for 20 min.
2. Cool the beaker in a tap water bath for 20 min.
3. Wash dishes with unheated 0.01 M citric buffer.
4. Wash dishes with de-ionized H₂O.
5. Store dishes in H₂O until use (preferably the same day).

LigandTracer measurement

1. Add approximately 3 ml PBS with 1 % BSA 15-30 minutes before placing the plate in the instrument. The BSA reduces the risk of non-specific binding.
2. Make sure to use instrument settings that are suitable for the number of tissue samples in the prepared dish. Choose any of the pre-defined templates in LigandTracer Control or define your own settings by unlocking an opened template.
3. Run measurement in PBS with 1 % BSA, e.g. as described in the protocol "A typical LigandTracer® measurement".