FISH HER2

1. SAMPLE: Tissue

2. Sample Pretreatment: Pretreatment procedure

Tissue - Slides pretreatment (sample releasing agent)

A. **Slides heating: Heat at 80ºC for 30min**

1. **BDW1**: Deparaffinization agent 68ºC for 15min.
2. **ETO2**: Wash with ETO2 at room temperature for 5min.
3. **HPE3**: ppe3 agent 90ºC for 20min.
4. **NDW4**: Wash with **NDW4** at 37ºC for 3min.
5. **IDIG5**: Digestion: Enzymic digestion at 37ºC for 10-40min. (30min)
6. **A-W6**: At room temperature, wash with cleaning solution twice, 5min each time.
7. **Dehydration7**: With 70%, 85% and 100% gradient EtOH for 2min.
8. Dry at room temperature.

3. DENATURATION & HYBRIDIZATION

Denaturation

Before **probe** use, invert the tube up and down for 5 times.

Centrifuge instantaneously for 1-2 sec

10 m probe add in slide.

Hybridization

A. FISH hybridization

a). 85ºC denaturation for 5min.

b). 42ºC hybridization for **2h**

4. Washing

1. Wash at room temperature with **2xSSC solution for 1min.**
2. Washing solution 68ºC (**NPS solution**) for **3min.**
3. Deionized water at 37ºC for 1min.
4. **Dry at room temperature.**

5. Dyeing

DAPI counterstaining for 10 min.

6. Results Reading