

## IS-PCR Accompanying Protocol: DNase Treatment

1. Execute standard deparaffinization, rehydration and PrK treatment (**steps 1-9 above**).
2. Incubate the tissue sections with Dnase in a humid chamber at 37°C O/N (~19hrs). I like to use the Boekel hybridization oven that accommodates the large humid chamber. Use 30-40 $\lambda$  of reaction mix to cover each prostate section. Use less for smaller tissues.

### Reaction Mix:

1X Reaction Buffer (supplied as 10X)

0.6U/ $\lambda$  DNase (1U/ $\lambda$  stock) In the future buy a more concentrated stock!

Sterile diH<sub>2</sub>O to volume.

3. Incubate in a large volume of **Rinse Solution** @RT for 10'  
(40 mM Tris-Cl pH 7.4, 2 mM CaCl<sub>2</sub> and 6mM MgCl<sub>2</sub>).
4. Rinse in multiple washes of sterile water (3) and let air dry for PCR.

## IS-PCR Recipes

### TBS (Tris-Buffered Saline)

100 mM Tris-Cl pH 7.4

150 mM NaCl

Autoclave sterilize.

Store at RT.

### Proteinase K Stock

Reconstitute lyophilized proteinase K to final concentration of 20 mg/mL, in a sterile

10 mM Tris pH 7.5, 20 mM CaCl, 50% glycerol solution, then make 500 $\lambda$  aliquots. This will enhance enzyme stability and allow repeated withdrawals from aliquots without diminishing the enzyme's activity. **DO NOT RECONSTITUTE IN WATER!**

Store at -20 °C.

### BSA V

There are two stocks, one for PCR and another for the blocking step.

Use a nuclease-free stock for PCR (ex. Gibco BRL)

For the blocking step, reconstitute BSA powder in TBS to a stock concentration of 200 mg/mL. Sterile filter and make 10 mL aliquots. Store both at -20 °C.

### CDB (Conjugate Dilution Buffer)

Make fresh--per mL add together:

100 $\lambda$  1M Tris pH 7.3

150 $\lambda$  1M MgCl<sub>2</sub>

50 $\lambda$  200 mg/mL BSA V.

700 $\lambda$  diH<sub>2</sub>O

### APSB (Alkaline Phosphatase Substrate Buffer)

100 mM Tris-Cl pH 9.5 A little trick: autoclave MgCl<sub>2</sub> separately from the Tris and salt to avoid precipitation.

150 mM NaCl

Store at 4°C to preserve alkaline pH.

50 mM MgCl<sub>2</sub>