

## 链霉亲和素-生物素标记免疫组化技术\*

### **Materials**

Cryosections of tissue specimen on glass slides

PBS

Primary antibody, ~5 to 10  $\mu\text{g}/\text{ml}$

Secondary antibody–fluorochrome conjugate specific to the source species of primary antibody

Mounting medium (e.g., Gelvatol)

Plastic slide box or moist chamber

Motorized pump or water pump

### **Set up slides**

**1. Layer wet paper towels in base of slide box to make a moist chamber, or make a chamber.**

**2. Remove slides with sections from cryostat or freezer and place across slide box (~6 per side) or in moist chamber so that the slides do not touch one another.**

**3. Once slides are at room temperature, but before they air dry, layer PBS over sections.**

*Do not flood the slides.*

### **Add primary antibody**

**4. Microcentrifuge diluted primary antibody 2 min at  $13,500 \times g$ ,  $4^{\circ}\text{C}$ .**

*A volume of 40 to 50  $\mu\text{l}$  antibody should cover the sections on each slide.*

**5. Remove PBS from slides by aspirating at one end of the sections with a Pasteur pipet connected to a pump, and introduce antibody at the other end.**

**6. Close box and incubate 1 hr at room temperature.**

**7. Wash slides three times for 5 min each in PBS.**

*For all washes, introduce new buffer at one end of sections and aspirate off old buffer from the opposite end.*

**8. Microcentrifuge diluted biotinylated secondary antibody (~40 to 50  $\mu\text{l}$  per slide) for 2 min at  $13,500 \times g$ ,  $4^{\circ}\text{C}$ .**

**9. Lay secondary antibody over sections and incubate in a moist chamber 1 hr at room temperature.**

**10. Wash slides three times for 15 min each in PBS.**

*For all washes, introduce new buffer at one end of sections and aspirate off old buffer from the opposite end.*

**11. Layer fluorochrome-streptavidin conjugate (~40 to 50  $\mu\text{l}$  per slide) over sections and incubate in a moist chamber for 1 hr at room temperature.**

**12. Wash slides three times for 5 min each in PBS.**

**13. Lay coverslips on paper towels and place a drop of Gelvatol in the middle of the coverslip.**

*If Gelvatol is not available, glycerol or PBS may be used for temporary mounting.*

**14. Invert slides on coverslips. Do not apply pressure, as this will damage the section; instead let the Gelvatol spread naturally.**

**15. Leave slides 30 min on bench under aluminum foil to keep out light and allow Gelvatol to harden.**

*Examine or store slides*

**16. Observe under microscope or store at 4°C in closed slide box.**

\*:出自 *Current Protocols in Molecular Biology* [B], *Wiley Online Library*,2007