PCR Blocker Protocol

PNA Storage and Handling

1. When ready to use, add 1 ml of sterile water to 50 nmole of each PNA tube or 500 ul to 25 nmole to make 50 uM stock. Vortex and mix well for complete dissolution. If particulate is still observed, heat at 60 °C for 10 minutes.
2. Aliquot PNA solution and store at – 20°C or – 70°C. Working stock can be diluted 10 times further (5 uM, 20x stock) and stored at 4 degrees for a couple of weeks.
3. When taking from cold storage, heat at 60°C for 10 minutes to ensure complete dissolution.
4. PNA is very stable as a lyophilized powder (>4 years) or as a solution in water (>2 years) if stored at – 20°C or lower. For long-term storage, – 70°C is recommended. PNA can go through several cycles of freeze and thaw.

PNA Clamping for PCR Reaction

1. For PCR in 50 ul, prepare the following PCR mix.
    a. 25 ul 2x PCR mix
    b. 2 ul PCR FW primer (5 uM stock) to final 0.2 uM
    c. 2 ul PCR RV primer (5 uM stock) to final 0.2 uM
    d. 2.5 ul mPNA blocker (5 uM stock) to final 0.25 uM
    e. 2.5 ul pPNA blocker (5 uM stock) to final 0.25 uM
    f. DNA template: 20 ng
    g. Water to 50 ul

2. Run PCR program as below.
    a. Denaturation: 94°C, 3 min
    b. Amplification (25~30 cycles)
        i. Denaturation: 94°C 15 sec
        ii. PNA clamping: 75°C for 10 sec
        iii. Primer annealing: 55~60°C for 10 sec
        iv. Extension: 68°C for 15~60 sec
    c. Extension: 72°C, 10 min
    d. Stored at 4°C

3. Take 5 ul of PCR product and run on the agarose gel.

4. The efficiency of PNA clamping (blocking PCR reaction) can vary depending on the amount of target and other conditions. In general, the more PNA oligo is added, the better the clamping effect is. To improve PNA blocking, mPNA and pPNA can be added up to 5 uM final concentration.

PNA Bio

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