

APPENDIX:

## **Sleeping Beauty and Prince Buffer**

**1) Fix in 200 mM Imidazole containing 0.1 % SDS:**

- 2.72 g Imidazole + 0.2 g SDS, dissolve in 200 mL dist. water.

*15 min with continuous shaking.*

**2) Rinse with dist water.**

**3) Stain (negative) with 200 mM Zinc sulphate:**

- 5.74 g Zn SO<sub>4</sub>, dissolve in 200 mL dist. water.

*Ca. 5 min with continuous shaking until white background has developed.*

**4) Rinse with dist water.**

**5) “Sleeping Beauty”**

Store in new fixing solution diluted 1 : 10 with dist. water

**6) “Prince”**

Mobilize proteins with 50 mM EDTA, 25 mM Tris; pH 8.3

- 0.61 g Tris + 3.72 g EDTA-Na<sub>2</sub>, dissolve in 200 mL dist. water, adjust to pH 8.3 with a few grains of Tris when necessary.

*6 min in 10 mL per strip with vigorous shaking.*

Continue with Equilibration of the strip.

**Ref.:**

Hardy E, Santana H, Sosa, Hernandez L, Fernandez-Patron C, Castellanos-Serra L. Imidazole-sodium dodecyl sulphate-zinc (reverse stain) on sodium dodecyl sulphate gels. Anal Biochem. 240 (1996) 150-152.