

TNP Conjugation to BRBC for Hemagglutination and plaquing

updated  
for BRBC

- REAGENTS: Cacodylate buffer pH 7.5  
 use Na cacodylate or cacodylate acid  
 $0.28M = 4.48g/100ml$  d H<sub>2</sub>O pH  $\approx$  50% NaOH  
 (2.24)/50ml  
 1X PBS pH 7.3  
 1X PBS + 1% glucose pH 7.3 10gms/litre  
 1X PBS + glycylglycine  
 (glycylglycine HCL -  $5.0 \times 10^3$  M  
 233mg/250ml 1X PBS to pH 7.2)

BRBC in Alsevsler's Sol.

PROCEDURE:

1. Wash BRBC 4X in PBS-glucose. 2000, 10 min
2. To 15ml packed BRBC, add 30 mg TNBS in 30 ml cacodylate buffer.
3. Mix gently in small Erlenmeyer for 10 min. at R.T..
4. ~~Add 1xPBS to 50 ml.~~
5. Spin in cold for 6 min. at 2000 rpm (Bodmer) (1EC)
6. Resuspend to 50 ml in 1xPBS glygly.
7. Let stand at room temperature for 10 min
8. Spin in cold 6 min
9. Wash 1X with 1X PBS glygly spin 10 min.
10. Wash 4X with 1X PBS-1% glucose
11. Resuspend in 50ml 1XPBS-glucose and refrigerate overnight

Cells should be prepared a day ahead for use in the Cunningham plaquing system. Spin down and resuspend cells to 5% in ISOMEM for use.

TNBS = Trinitrobenzenesulfonic acid, bought commercially Eastman Organic.

2x/8x 25/1  
 108/6.1  
 110/8.0  
 111/11.1  
 111/11.1  
 112/11.1  
 113/11.1  
 114/11.1  
 115/11.1  
 116/11.1  
 117/11.1  
 118/11.1  
 119/11.1  
 120/11.1  
 121/11.1  
 122/11.1  
 123/11.1  
 124/11.1  
 125/11.1  
 126/11.1  
 127/11.1  
 128/11.1  
 129/11.1  
 130/11.1  
 131/11.1  
 132/11.1  
 133/11.1  
 134/11.1  
 135/11.1  
 136/11.1  
 137/11.1  
 138/11.1  
 139/11.1  
 140/11.1  
 141/11.1  
 142/11.1  
 143/11.1  
 144/11.1  
 145/11.1  
 146/11.1  
 147/11.1  
 148/11.1  
 149/11.1  
 150/11.1