

TNP Conjugation to SRBC for Hemagglutination
AND PLAQUING

Reagents: Cacodylate buffer pH 7.5 Na Cacodylate STORED IN DESICCATOR IN COLD AFTER OPENING
or cacodylic acid
(both forms available)

0.28M = 4.48g/100 ml disp H₂O
pH \approx 50% NaOH \approx 3ml
(2.24g/50 ml)

1X PBS pH 7.3 make from 10X PBS + pH \approx 5N NaOH

1XPBS + 1% glucose pH 7.3

1XPBS - glycylglycine

(glycylglycine·HCl - 5.0×10^{-3} M \rightarrow 233 mg/250 ml 1X PBS to pH 7.2)

SRBC in Alsever's

(925mg/100ml)

Procedure

1. Wash SRBC 4X in PBS-glucose 10ml
2. To 1 ml packed SRBC, add 20 mg TNBS in 20 ml cacodylate buffer
3. Mix gently in small Erlenmeyer for 10 min at R.T. 1:100
(50 ml)
4. Add 1X PBS to 50 ml
5. Spin in cold for \sim 10 min at 2000 rpm (Bedmer)
6. Resuspend to 50 ml in 1XPBS glygly
7. Let stand at R.T. for 10 min
8. Spin in cold
9. Wash 1X with 1XPBS glygly
10. Wash 3X with 1XPBS-1% glucose
11. Resuspend in 20 ml ISOMEM to make a 5% solution for Cunningham Plaquing.
Use within 24 hours

These cells are used in α DNP plaquing system and if prepared the evening before use, give the same number of developed and direct plaques as DNP-alanylglycylglycine -SRBC
ala Merchant

TNBS = Trinitrobenzenesulfonic acid, bought commercially. Eastman Organic

Prepare TNBS'd SRBC the day before needed in order to assure non-hemolysis of cells. Much variation among sheep cell sensitivity to TNBS. Perhaps older SRBC (2-3 weeks) are less sensitive to spontaneous lysis.