

Preparation of KLH, HGG coated Gluteraldehyde Fixed SRBC for Hemagglutination

1. Wash fresh SRBC (Alsevers) 3X with 1X PBS.
2. After final wash is decanted make up 50% solution of SRBC using 0.15 M phosphate buffer pH 7.3

0.15M Phosphate Buffer : 1) $0.15M Na_2HPO_4$ (5.3g \rightarrow 250 ml d. H₂O)
 e. $0.15M KH_2PO_4$ 13.4g \rightarrow 250 ml d. H₂O

2) $0.15 M KH_2 PO_4$ (5.1 g \rightarrow 250 ml d H₂O)

For pH 7.3: 47 ml of(2) + 153 ml of (1)

3. For each 0.1 ml of 50% cell suspension:

add 10 mg KLH or HGG in 3.2 ml 0.15M phosphate buffer.
 incubate cells and Ag solution for 1/2 hr at 37°C (water bath)
 for every 0.1 ml of ^{SRBC suspension} suspension add 1 ml of .125% v/v gluteraldehyde
 in .15 M PO₄ buffer (dropwise addition with agitation)
 return to 37°C water bath for 1 hr.
 wash coated-fixed cells 2X with 0.15 M phosphate buffer
 resuspend to 2% suspension for hemagglutination

Cells last 1 month at 4°C (no preservatives)

Supplies: see KLH procedure

Hgg crystalline, purchased from Pentex