

WHOLE B-CELL ELISA

Supplies and Reagents

- FACS buffer – 1% BSA in DPBS
- Substrate buffer
 - 1.69g NaHCO₃
 - 2.51g Na₂CO₃
 - 0.41g MgCl₂ or 1.39g MgCl₂•6H₂O
 - H₂O to 1950mL
 - Adjust pH to 8.6 with NaOH or HCl, then add H₂O to 2 liters
- Goat αMouse IgM-AP from Southern Biotech (Cat. # 1021-04)
- P-nitrophenyl phosphate (AMRESCO #0405-100T)

I. First Washes and Antibody Incubation

1. Plate cells in desired concentrations in volumes up to 200μL in flat bottom 96 well plates.
2. Spin the cells in a centrifuge at 1000rpm for 5 minutes. To remove the media, flick the plate with medium force over a sink. Keeping the plate turned upside down after flicking, gently blot the well-side on a pad of paper towels three times to remove any media that remained on the plate.
3. Perform two FACS buffer washes with 200μL per well, flick the plates as described above for these washes.
4. Add 50μL of 1:2000 dilution IgM-AP to each well. Incubate on ice for one hour.

II. Second Washes and PNPP

1. Add 150μL of FACS buffer to the contents of all wells for the first wash. Perform two more 200μL FACS washes to ensure that all excess antibody has been removed. These washes should be done in the manner previously described.
2. Add 100μL of PNPP at 1mg/mL substrate buffer to all wells. Incubate plates at 37°C for fifty minutes to one hour.
3. Read OD at 405nm.