Mishell-Dutton culture methods.

-Flick out antigen from the plates and block non-specific sites with 3% BSA/TBS. Do not use tween prior to cell incubation. Incubate at RT for one hour.

-Remove cells from 24 well cluster by gently scraping with a 5 ml pipette. Triplicate cultures (1.5 ml/culture) are added together and centrifuged at 1800 rpm (GLC-2) for 5 minutes. Supernatant is removed and cells are washed with BSS. Resuspend cells to original volume in ELISPOT media.

-Wash plates with BSS once. Flick dry , then add cells. Be fast at this step and do not let media turn purple. Culture cells for 4-5 hours at 5% CO_2, 37 C. Use an incubator that will not be used during this time since opening and closing the door will cause cells to move around on the plate.

-Remove cells from the incubator. Observe under the inverted scope to assess for crowding in the wells and to follow the effectiveness of washes. Wash 5-6 times with TBS/Tween.

-Add antibodies in 3% BSA/TBS/Tween.
IgG- Southern Biotech (SBA), Goat anti-Mouse IgG (gamma chain specific) 1.0 mg/ml. use at 1:500.
IgM-SBA, Goat anti-Mouse IgM, Mu chain specific, Alkaline phosphatase labeled. (1:1000)

Incubate at 4 C overnight.

-IgG plates. Wash 3 times with TBS/Tween, Add Swine anti-Goat (H+L chain specific), alkaline phospatase labeled (TAGO) in 3% BSA/TBS/Tween at 1:1000. Incubate 5-6 hours at RT or overnight at 4C.

- Development: prepare development buffer as previously described once it has been equilibrated to 65 C with agarose wash the plates three times. Flick dry and add development buffer at 100 ul per well. Use a flat, immobile surface. Let stand at RT for 20 minutes to allow agarose to gel. Do not move during this time. Then place in 37 C and within 2-3 hours maximal spot development will occur.

-Count AFC/well using low power on an inverted scope.