

Date Run _____
Experiment _____
File Name _____

Antigen: Whole Milk
1° antibody & ratio: SAC, 1:500 (16µl in 8.0ml)
2° antibody & ratio: RASheep, 1:1500 (5.4µl in 8.1ml)
Plate Type: MaxiSorp (Nunc 456537)

Coating Buffer: TBS + 1.1% EDTA (wt/vol)

Blocker: PBS + 20% Bovine Serum (PBS-BS)

Detergent: PBS + 0.09% Triton X-100 (PBST) & PBS + 2.3g/L SDS (PBS-SDS)

1° Antibody Diluent: PBS +1300 ppm Silwet + 20% Bovine Serum (PBSS-BS 20)

2° Antibody Diluent: PBS +1300 ppm Silwet + 20% Bovine Serum (PBSS-BS 20)

Substrate: ultra TMB (Pierce 34028)

Protocol (Indirect ELISA) Casein Assay

1. Add 80µl of samples to wells and incubate 120 min @ 37°C
2. Wash plate 5x with PBST (300 µl/well)
3. Add 300 ul of blocker to wells, incubate for 60 min @ 37°C
4. Wash plate 2x with PBST (300 µl/well)
5. Add 80µl 1° antibody/ well, incubate for 30 min @ 37°C
6. Hand wash plate 2x with PBST (300 µl/well), then 3x in plate washer (300 µl/well)
7. Add 80 µl 2° antibody/ well, incubate for 120 min @ 37°C
8. Wash plate 3x with PBS-SDS (300 µl/well)
9. Wash plate 3x with PBST (300 µl/well)
10. Add 80µl substrate /well & incubate 10 minutes, RT, dark, rotating
11. Stop with 80µl 2N H₂SO₄ /well & read at 450 - 490 nm

Notes:

SAC = Sheep anti-casein
(BioDesign K20025S)
RASheep = Rabbit anti-sheep
(Pierce 31480)

Purpose of Experiment:

Plate Layout:

