

Date Run \_\_\_\_\_

Experiment \_\_\_\_\_

File Name \_\_\_\_\_

Antigen: Soy Milk

1° antibody & ratio: RASoy, 1:2000 (4µl in 8.0ml)

2° antibody & ratio: DAR, 1:10,000 (1µl in 10.0ml)

Plate Type: MaxiSorp (Nunc 456537)

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

Blocker: PBS +1300 ppm Silwet + 20% Bovine Serum (PBSS-BS 20)

Detergent: PBS + 0.09% Triton X-100 + 2% PEG (PBST-PEG) &  
PBS + 2.3g/L SDS + 2% PEG (PBS-SDS-PEG)

1° Antibody Diluent: PBS +1300 ppm Silwet + 30% Bovine Serum (PBSS-BS 30)

2° Antibody Diluent: PBS +1300 ppm Silwet + 30% Bovine Serum (PBSS-BS 30)

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-PEG (300 µl/well)
3. Add 300 ul of blocker to wells, incubate for 60 min @ 37°C
4. Wash plate 2x with PBST-PEG (300 µl/well)
5. Add 80µl 1° antibody/ well, incubate for 30 min @ 37°C
6. Hand wash plate 2x with PBST-PEG (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-PEG (300 µl/well)
9. Wash plate 3x with PBST-PEG (300 µl/well)
10. Add 80µl substrate /well & incubate 5 minutes, RT, dark, rotating
11. Stop with 80µl 2N H<sub>2</sub>SO<sub>4</sub> /well & read at 450 - 490 nm

### Notes:

RASoy = Rabbit anti-soy  
(r-biopharm R45254)  
DAR = Donkey anti-rabbit  
(Pierce 31458)

### Purpose of Experiment:

### Plate Layout:

