INDIRECT ELISA

1. Coating with antigen: 100 µl/well of antigen (Hydatic Fluid: 5 µg/ml; Purified Recombinant Protein: 0,5 µg/ml) in carbonate buffer 16 hr. at 4 ^{o}C (O/N at 4 ^{o}C), or 1 hr at 37 ^{o}C.
2. Washes: 200 µl/well of wash buffer (TPBS), room temperature, 5 times. Machine
3. Post-coating: 200 µl/well of wash buffer + 1% BSA, 1 hr at 37 ^{o}C (optional, prevent high backgrounds)
4. Washes as in step No. 2
5. Sera: 100 µl/well of serum (1/200) diluted in wash buffer + 1% BSA, 1 hr at 37 ^{o}C.
6. Washes as in step No. 2
7. Anti-human IgG Peroxidase conjugated: 100 µl/well at 1/2000 dilution in wash buffer + 1% BSA, 1 hr at 37 ^{o}C.
8. Washes as in step No. 2
9. Substrate: 100 µl/well of substrate solution, incubate at room temperature in darkness
    - 0.0028 g OPD (orto-fenilen-diamine)
    - 10 ml of OPD buffer, pH 5
    - 3 µl de H_2O_2 33%
10. Stopping: with 50 µl/well of 3N H_2SO_4.
11. Reading absorbance at 492 nm.

REAGENTS

✓ Carbonate Buffer

34 mM $NaHCO_3$	2.90 g
27 mM Na2CO3	1.59 g
Take up to 900 ml with H2O type II	
Adjust pH 9.6	
Adjust to 1L H2O type II	

✓ Wash buffer

PBS 1 x	1 L
Tween20, 0.05%	500 µl

✓ Citrate Buffer or OPD buffer

25 mM citric acid	5.25 g
25 mM Na2HPO4•12 H2O	3.54 g
Take up to 900 ml with H2O type II	
Adjust pH to 5	
Adjust to 1L H2O type II	