

Anti-BPI protein/peptide ELISA Protocol

Day #1

1. Coat each well with 100ul Protein/Peptide (10ug/ml, diluted in PBS) or PBS alone
2. Incubate for 2hr at 37C
3. Wash 3X with PBS + 0.05% Tween
4. Add 300ul PBS+5% Normal Goat Serum (NGS) and incubate o/n at RT

Day #2

5. Prepare patient sera 1:100 in PBS+1% NGS
6. Wash coated ELISA plate 3X with PBS + 0.05% Tween
7. Add 100ul patient serum sample to appropriate wells
8. Incubate for 1hr at RT
9. Wash 3X with PBS + 0.05% Tween
10. Add 100ul goat-anti-human HRP-labeled 2' ab (1:50,000 dil. in PBS+1% NGS) and incubate for 1hr at RT; take out Substrate Reagents and Stop soln. to warm up at RT, keep in the dark.
11. Wash 3X with PBS + 0.05% Tween
12. Add 100ul substrate (R&D, Substrate Reagent A: Substrate Reagent B 1:1)
13. Incubate at RT in the dark for ~15min
14. Add 50ul stop solution and read absorbance at 450nm and 570nm (for correction).