1. Coat wells of a 96-well microtiter plate with 200 ul of 2 ug/ml laminarin in PBS (pH 7.0), and incubate for 16 hours at 4˚C.
2. Wash wells 3 times with PBS containing 0.05% Tween-20 (PBST).
3. Block wells with 300 ul 0.5% gelatin in PBST (PBTG) at 37˚ for 30 minutes.
4. Remove blocking solution. Add 100 ul of standard or sample to wells, and 100 ul of 1˚ Ab diluted 1:100,000 in PBGT and mix. Shake plate at 37˚ for 1.5 hours.
5. Wash wells 3 times with PBST.
6. Add 200 ml anti-mouse IgG peroxidase labeled antibody diluted 1:5,000 to each well. Shake plate for 1 hour at 37˚C.
7. Wash wells 3 times with PBST.
8. Add 200 ul of o-phenylenediamine (2 mg/ml) in 0.05 citrate-phosphate buffer, pH 5.5, containing 0.015% (v/v) hydrogen peroxide to wells and incubate for 30 minutes at 20˚C.
9. Terminate the reaction by adding 50ul of 2M HCl.
10. Measure absorbance at 490 nm.