

A β ELISA Protocol

A β can be detected in tissue culture supernatant and in homogenized tissue. Two anti-A β antibodies will be needed, one for coating onto the ELISA plate for capture and a secondary biotinylated antibody for detection.

1. Coat ELISA 96-well plate with 100 μ l per well of antibody (at a final concentration of 3.5 μ g/mL) diluted in Well-Coating Buffer. Well-coating buffer may need to be warmed at 37°C to bring it into solution.
-Per plate (96 wells), make 10 mL antibody in Well-Coating Buffer.
2. Cover with plate sealer (tape interface to prevent evaporation) and incubate O/N at RT.
3. The next morning, aspirate Well-Coating Buffer.
4. Add 200 μ l per well Well Fixing Buffer.
5. Seal and incubate \geq 1 hr at RT.
6. Aspirate Well Fixing Buffer.
7. Add 100 μ l/well standards, controls and unknowns. Use synthetic A β (either 1-40 or 1-42) standards, diluting serially from 1,000 pg/mL to 2 pg/mL in each ELISA plate.
8. Seal and incubate \geq 1hr at RT.
9. Aspirate supernate.
10. Wash 3 x 1 min with TBS wash buffer.
11. Add 100 μ l/well biotinylated secondary antibody (the optimal antibody concentration should be determined empirically after biotinylation) diluted in Specimen Diluent.
12. Seal and incubate 1hr at RT.
13. Aspirate biotinylated antibody.
14. Wash 3 x 1 min with TBS wash buffer.
15. Add 100 μ l/well HRP-Avidin D diluted 1:2000 in Specimen Diluent.
16. Seal and incubate 1 hr at RT (begin warming substrate).

17. Aspirate supernate.
18. Wash 3 x 1 min with TBS wash buffer.
19. Add 100 µl/well QuantaBlu (warmed to RT), mixed 9 parts Substrate Solution with 1 part Stable Peroxide Solution.
20. Seal and incubate 1 hr at RT. Check the reaction development with UV light, if desired.
21. Add 100 µl/well Stop Solution and mix by gently shaking the plate.
22. Read on a fluorometer, (excitation 340, emission 400).

Reagents

<i>ELISA Plate</i>	FluoroNunc MaxiSorp, high binding Flat bottom, no lid.
<i>HRP-Avidin D</i>	Vector Labs A-2004 5mg/ml
<i>QuantaBlu</i>	Pierce #15169

Well Coating Buffer (1 L)

Sodium phosphate monobasic(monohydrate)	0.23g
Sodium phosphate dibasic	26.2g
Sodium azide	1.0g
MilliQ H2O	1 L

Caution: sodium azide releases poisonous gas at acidic pH

1. Dissolve phosphates in 950 mL H2O, stir well.
2. Adjust pH to between 8.3 and 8.7.
3. Add sodium azide.
4. Add water to final volume.
5. Stir well and filter through 0.2 µm membrane.
6. Store 4°C.

0.25% Well Fixing Buffer

Sucrose, crystalline	25g
Sodium phosphate dibasic	10.8g
Sodium phosphate monobasic	1.0g
Sodium azide	0.5g
HSA, 30% solution (Sigma A-6909)	8.33 mL (BSA may substitute)
MilliQ H2O	992 mL

1. Dissolve phosphates and sucrose in 950 mL H₂O.
2. Adjust pH to between 7.2 and 7.6.
3. Add sodium azide.
4. Add HAS and stir until dissolved.
5. Add water to final volume.
6. Stir well and filter through 0.2 µm membrane.
7. Store 4°C.

Specimen Diluent

Sodium phosphate monobasic	0.2g
Sodium phosphate dibasic	2.16g
Thimerosal	0.5g
Sodium chloride	8.5g
Triton x-405	0.5mL
BSA, globulin free (Sigma A-7030)	6.0g
MilliQ Water	1L

Caution: Thimerosal is poisonous.

1. Dissolve phosphates, Thimerosal, and NaCl in 950 mL H₂O.
2. Add triton X-405, rinsing pipette well.
3. Add BSA and stir well.
4. Adjust pH to between 7.3 and 7.5.
5. Add water to final volume, stir well.
6. Sterile filter through 0.2 µm membrane.
7. Store 4°C.

10X TBS, pH 7.5 at 1x

Sodium chloride	80.0g
Potassium chloride	3.8g
Tris base	5.85g
Tris HCl	31.75g
MilliQ H ₂ O	1 L

1. Dissolve salts in 950 mL H₂O.
2. Make a 1X dilution with 1 mL of 10x in 9 mL H₂O. pH should be between 7.5 and 7.8. Adjust 10X solution until diluted TBS is correct.
3. Sterile filter through 0.2 µm membrane.
4. Store 4°C.

TBS Wash Buffer - TBS-T 0.05% Tween 20, pH 7.5

20X TBS (pH 7.5 at 1X)	50 mL
Tween 20	0.5 mL
MilliQ H2O	949.5 mL

1. Combine ingredients and mix well.

Antibodies should be tested for their compatibility with the ELISA assay. For this protocol, the following antibodies have been used successfully:

<u>Antibody</u>	<u>Epitope</u>	<u>Source</u>
2G3	Abeta 40 C-terminus	Elan Pharmaceuticals
21F12	Abeta 42 C-terminus	Elan Pharmaceuticals
266	Abeta residues 13-28	Elan Pharmaceuticals
3D6	Abeta 1-5 N-terminus	Elan Pharmaceuticals