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Title: Standard Operating Procedure (SOP) for Quantification ELISA BUFFERS

Introduction:

The IgG level is determined by an indirect ELISA method using a single serum / plasma dilution obtained by checker-board titration experiments. The optical density (OD) of the test sample is converted into arbitrary units (A.U.) by means of a standard-curve from known concentrations of purified human polyclonal IgG.

Wells of microtiter plates are coated with either GLURP₂₅₋₅₁₄ antigen or serial dilutions of the purified human polyclonal IgG. Diluted test-samples and controls (primary antibody) are added to the wells containing the GLURP₂₅₋₅₁₄ antigen and specific antibodies against GLURP are revealed by Peroxidase conjugated rabbit anti-human IgG (secondary antibody). Bound secondary antibody is quantified by colouring with ready to use TMB (3,3', 5,5'-Tetramethylbenzidine) substrate. Optical density (OD) is read at 450 nm with a reference at 620nm in a plate reader, and the OD value of the test-sample is converted into A.U. by means of a standard curve on each plate.

ELISA protocol involves the use of different kinds of reagents and buffers, some of which have to be prepared fresh or some few days before they are used. This document describes how these reagents are prepared, their storage conditions and their shelf life.

1. COATING BUFFER

(1x PBS)

Volume: 1L

PROCEDURE:

1. Weigh 0.1 g of phenol red and place it in a small beaker or tube. Add 10 ml of deionised water to the phenol to make 1% phenol red solution
2. Add 2 tablets of PBS to a beaker containing 1000ml deionised water.
3. Place the flask on a magnetic stirrer without heating and stir until all is in solution.
4. Add 1 ml of a 1 % Phenol red solution.
5. Label: "Coating buffer", preparation date, initials of the preparing personnel and the date beyond which the buffer should not be used.
6. Store at 2 – 8 °C for up to 3 months

2.BLOCKING BUFFER

(PBS, 3% milk powder,0.1 % Tween-20)

Volume: 1L

1. Add 2 tablets of PBS to a beaker containing 1000ml deionised water.
2. Place the flask on a magnetic stirrer without heating and stir until all is in solution.
3. Add 30 g of skimmed milk
4. Add 1 ml of Tween-20 and continue stirring until all is in solution
5. Label: "Blocking buffer", preparation date and initials of the preparing personnel

USE FRESH BLOCKING BUFFER EACH TIME!!

3. SERUM DILUTION BUFFER

(PBS, 1% milk powder,0.1 % Tween-20 and 0.02% Na-az)

Volume: 1L

1. Weigh 4 g Na-azide and place it in a small beaker. Add 40 ml of deionised water to the Na-az to make 10% Na-az solution
2. Add 2 tablets of PBS to a beaker containing 1000ml deionised water.
3. Place the flask on a magnetic stirrer without heating and stir until all is in solution.
4. Add 10 g of skimmed milk
5. Add 1 ml of Tween-20
6. Add 2ml of 10% Na-az solution and continue stirring until all is in solution
7. Label: "Serum Dilution buffer", preparation date, initials of the preparing personnel and the date beyond which the buffer should not be used.
8. Store at 2 – 8 °C for up to 3 weeks

4. WASHING BUFFER

(PBS, 0.1% Tween-20 and 0.5 M NaCl)

Volume: 5L

1. Add 10 tablets of PBS to a beaker containing 5000ml deionised water.
2. Place the flask on a magnetic stirrer without heating and stir until all is in solution
3. Add 145g of NaCl
4. Add 5ml of Tween-20 and continue stirring until all is in solution
5. Label: "Washing buffer", preparation date, initials of the preparing personnel and the date beyond which the buffer should not be used.
6. Store at Room Temperature for up to 1 week

5. DILUTION BUFFER

(PBS, 1% milk powder and 0.1% Tween-20)

Volume: 1L

1. Add 2 tablets of PBS to a beaker containing 1000ml deionised water.
2. Place the flask on a magnetic stirrer without heating and stir until all is in solution.
3. Add 10 g of skimmed milk
4. Add 1 ml of Tween-20 and continue stirring until all is in solution
5. Label: "Dilution buffer", preparation date, initials of the preparing personnel and the date beyond which the buffer should not be used.
6. Store at 2 – 8 °C for up to 1 week

6. STOP SOLUTION

(0.2 M H₂ SO₄)

Volume: 1L

1. Add 980 ml of deionised water to a 1000ml flask
2. Add 20 ml of 10 M H₂ SO₄
3. Cool to room temperature
4. Label: "0.2 M H₂ SO₄", preparation date, initials of the preparing personnel and the date beyond which the buffer should not be used.
5. Store at Room temperature for up to 8 months

6. COLOUR SOLUTION

(TMB [3,3',5,5'- Tetramethylbenzidine)

Volume: 120 ml for 12 plates

This comes already prepared as a *ready-to-use* solution

1. Transfer the amount needed into 50 ml centrifuge tubes covered with aluminium foil
2. Label "TMB Colour solution"
3. STORAGE: TMB solution is kept at 4 °C, however the solution for immediate use must be brought to room temperature

References:

Dodoo, D., M. Theisen, J. A. Kurtzhals, B. D. Akanmori, K. A. Koram, S. Jepsen, F. K. Nkrumah, T. G. Theander, and L. Hviid. 2000. Naturally acquired antibodies to the glutamate-rich protein are associated with protection against *Plasmodium falciparum* malaria. *J.Infect.Dis.* 181:1202-1205.

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