Biochemical analysis of quail blood

Gamal Mehaisen,Ahmed O. Abass

Abstract

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Protocol

Sample preparation:

Step 1.

1. Blood samples were collected into heparinized tubes.
2. Samples were centrifuged at 2000 xg for 10 min at 4º C.
3. The plasma was separated and stored at -20 ºC until analyzed.

Lipid peroxidation (Colorimetric MDA Assay Kit, ab118970, Abcam, UK):

Step 2.

1. Add 600 µL of Thiobarbituric Acid (TBA) solution to 200 µL standard and 200 µL test samples.
2. Incubate TBA-standard/TBA-sample mixture at 95 °C for 60 minutes.
3. Cool to room temperature in an ice bath for 10 minutes.
4. Pipette 200 µL from each 800 µL TBA-standard and TBA-sample reaction mixture into a 96 well microplate.
5. Measure plate immediately at OD532 nm for colorimetric assay.

Alanine aminotransferase (Colometric ALT Assay Kit, Ref-264, Spectrum Diagnostics, Egypt):

Step 3.

1. Add 0.5 mL of R1 (100 mmol Phosphate buffer, 200 mmol DL-Alanine, 6 mmol 2-Oxoglutarate, and 12 mmol Sodium Azide) to 100 µL of distilled water or test samples.
2. Mix and incubate for exactly 30 minutes at 37 °C.
3. Add 0.5 mL of R2 (2,4-dinitrophenyl hydrazine) to all tubes.
4. Mix and incubate for exactly 20 minutes at 20-25 °C.
5. Mix with 0.5 mL of sodium hydroxide (0.4 mol/L).
6. Measure absorbance of samples against reagent blank at 546 nm after 5 minutes.
7. The sensitivity of this assay is 4 U/L and the analytical range is 4-94 U/L.

Asparate aminotransferase (Colometric AST Assay Kit, Ref-260, Spectrum Diagnostics, Egypt):

Step 4.

1. Add 0.5 mL of R1 (100 mmol Phosphate buffer, 100 mmol L-aspartate, 5 mmol 2-Oxoglutarate, 140 mmol sodium hydroxide, and 12 mmol Sodium Azide) to 100 µL of distilled water or test samples.
2. Mix and incubate for exactly 30 minutes at 37 °C.

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3. Add 0.5 mL of R2 (2 mmol 2,4-dinitrophenyl-hydrazine and 8.4 % HCl) to all tubes.
4. Mix and incubate for exactly 20 minutes at 20-25 °C.
5. Mix with 0.5 mL of sodium hydroxide (0.4 mol/L).
6. Measure absorbance of samples against reagent blank at 546 nm after 5 minutes.
7. The sensitivity of this assay is 7 U/L and the analytical range is 7-89 U/L.

Triglycerides (GPO-PAP-enzymatic colorimetric Assay Kit, Ref-314, Spectrum Diagnostics, Egypt):

Step 5.

1. Add 1.0 mL of prepared Reagent to 10 µL of standard triglyceride (200 mg/dl) or test samples.
2. Mix and incubate for 5 minutes at 37 °C.
3. Measure absorbance of samples (A_{sp}) and standard (A_{st}) against reagent blank at 546 nm within 30 minutes.
4. Triglycerides conc. (mg/dL) is calculated as (A_{sp}/A_{st}) x 200.

Cholesterol (CHOD-PAP-enzymatic colorimetric Assay Kit, Ref-230, Spectrum Diagnostics, Egypt):

Step 6.

1. Add 1.0 mL of prepared Reagent to 10 µL of standard cholesterol (200 mg/dl) or test samples.
2. Mix and incubate for 5 minutes at 37 °C.
3. Measure absorbance of samples (A_{sp}) and standard (A_{st}) against reagent blank at 546 nm within 30 minutes.
4. Cholesterol conc. (mg/dL) is calculated as (A_{sp}/A_{st}) x 200.

Calcium (O-CPC colorimetric Assay Kit, Ref-226, Spectrum Diagnostics, Egypt):

Step 7.

1. Mix 0.5 mL of R1 (0.3 mol 2-Amino-2-methyl-1-propanol, pH 10.5) and 0.5 mL of R2 (0.16 mmol O-cresolphthalein complexone, 7 mmol 8-hydroxyquinoline).
2. Add the mixture to 10 µL of standard calcium (10 mg/dl) or to 10 µL of test samples.
3. Incubate for 5 minutes at 20-25 °C.
4. Measure absorbance of samples (A_{sp}) and standard (A_{st}) against reagent blank at 578 nm.
5. Calcium conc. (mg/dL) is calculated as (A_{sp}/A_{st}) x 10.

Phosphorus (UV colorimetric Assay Kit, Ref-294, Spectrum Diagnostics, Egypt):

Step 8.

1. Add 1.0 mL of Reagent (3.5 mmol ammonium molybdate, 750 mmol sulphuric acid, and 1% Surfactants) to 10 µL of either blank reagent (distilled water), standard reagent (5 mg/dl phosphorus) or test samples.
2. Mix and wait for 5 minutes at 37 °C.
3. Measure absorbance of samples (A_{sp}) and standard (A_{st}) against reagent blank at 340 nm within 30 minutes.
4. Phosphorus conc. (mg/dL) is calculated as (A_{sp}/A_{st}) x 5.

Total protein (Biuret colorimetric Assay Kit, Ref-310, Spectrum Diagnostics, Egypt):

Step 9.

1. Add 1.0 mL of Reagent (750 mmol sodium hydroxide, 12 mmol copper sulphate, 40.9 mmol sodium potassium tartrate, and 19.8 mmol potassium iodide) to 20 µL of either standard total protein (6 mg/dL) or test samples.

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2. Mix and incubate for 10 minutes at room temperature.
3. Measure absorbance of samples (A_{sp}) and standard (A_{st}) against reagent blank at 546 nm within 30 minutes.
4. Protein conc. (mg/dL) is calculated as (A_{sp}/A_{st}) x 6.

Albumin (BCG colorimetric Assay Kit, Ref-211, Spectrum Diagnostics, Egypt):

Step 10.

1. Add 1.0 mL of Reagent (100 mmol acetate buffer, 0.27 mmol Bromocresol green, and detergent) to 10 µL of either standard albumin (4 g/dL) or test samples.
2. Mix and incubate for approximately 5 minutes at 20-25 °C.
3. Measure absorbance of samples (A_{sp}) and standard (A_{st}) against reagent blank at 623 nm within 60 minutes.
4. Protein conc. (mg/dL) is calculated as (A_{sp}/A_{st}) x 4.

Corticosterone (Chicken CORT ELISA Kit, MBS701668, MyBioSource Inc., USA):

Step 11.

1. Add 50 µL of standard and sample per well.
2. Add 50 µl Antibody to each well immediately.
3. Mix well with the pipette for 30 seconds and cover with the adhesive strip provided.
4. Incubate for 30 minutes at 25 °C.
5. Aspirate each well and wash with Wash Buffer (250µl) using a multi-channel pipette.
6. Repeat the process three times for a total of four washes.
7. After the last wash, remove any remaining Wash Buffer and blot the plate inversly against clean paper towels.
8. Add 100 µL HRP-conjugate to each well immediately and cover with the adhesive strip provided.
9. Incubate for 30 minutes at 25°C.
10. Repeat the aspiration/wash process for four times as in step 5.
11. Add 100 µL of TMB Substrate to each well.
12. Incubate for 15 minutes at 25°C, protecting from light.
13. Add 50 µL of Stop Solution to each well and gently tap the plate to ensure thorough mixing.
14. Determine the optical density of each well within 5 minutes, using a microplate reader set to 450 nm, 540 nm or 570 nm.
15. Subtract readings at 540 nm or 570 nm from the readings at 450 nm.

Tumor necrosis factor alpha (Chicken TNF-α ELISA Kit, MBS701522, MyBioSource Inc., USA):

Step 12.

1. Set a Blank well without any solution.
2. Add 50 µL of standard and sample per well.
3. Add 50 µL HRP-conjugate (1x) to each standard/sample wells immediately.
4. Mix well with the pipette for 60 seconds and cover with the adhesive strip provided.
5. Incubate for 40 minutes at 37 °C.
6. Aspirate each well and wash with Wash Buffer (250µl) using a multi-channel pipette.
7. Repeat the process three times for a total of four washes.
8. After the last wash, remove any remaining Wash Buffer and blot the plate inversly against clean paper towels.
9. Add 90 µL of TMB Substrate to each well.
10. Incubate for 20 minutes at 37 °C, protecting from light.

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11. Add 50 µL of Stop Solution to each well and gently tap the plate to ensure thorough mixing.
12. Determine the optical density of each well within 5 minutes, using a microplate reader set to 450 nm, 540 nm or 570 nm.
13. Subtract readings at 540 nm or 570 nm from the readings at 450 nm.

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