

ELISA kits available from ADI (see details at the web site)

| | |
|--------------------|---|
| VAC-DTX-50 | VacciGel Direct ELISA for the measurement of Diphtheria Toxoid in Vaccines formulated in Alum, 96 tests |
| VAC-DTX-210 | Diphtheria Toxoid/Toxin (DTX) ELISA for the measurement DTX in biological buffer |
| VAC-HBS-50 | VacciGel Direct ELISA for the measurement of Hepatitis B Vaccine (HBsAg) formulated in Alum, 96 tests |
| VAC-HCG-50 | VacciGel Direct ELISA for the measurement of HCG (contamination) in Vaccines formulated in Alum, 96 tests |
| VAC-PTX-50 | VacciGel Direct ELISA for the measurement of Pertussis Toxoid in Vaccines formulated in Alum, 96 tests |
| VAC-TTX-410 | Pertussis Toxoid/Toxin (PTX) ELISA for the measurement PTX in biological buffer |
| VAC-TTX-50 | VacciGel Direct ELISA for the measurement of Tetanus Toxoid in Vaccines formulated in Alum, 96 tests |
| VAC-DTX-310 | Tetanus Toxoid/Toxin (PTX) ELISA for the measurement PTX in biological buffer |
| 930-100-TTH | Human Anti-Tetanus Toxin/Toxoid IgG ELISA kit, 96 tests, Quantitative |
| 930-110-TTM | Mouse Anti-Tetanus Toxin/Toxoid Ig's (G+A+M) ELISA kit, 96 tests, Quantitative |
| 930-120-TMA | Mouse Anti-Tetanus Toxin/Toxoid IgA ELISA kit, 96 tests, Quantitative |
| 930-130-TMG | Mouse Anti-Tetanus Toxin/Toxoid IgG ELISA kit, 96 tests, Quantitative |
| 930-140-TMM | Mouse Anti-Tetanus Toxin/Toxoid IgM ELISA kit, 96 tests, Quantitative |
| 930-200-TTR | Rabbit Anti-Tetanus Toxin/Toxoid Ig's (G+A+M) ELISA kit, 96 tests, Quantitative |
| 930-210-TRG | Rabbit Anti-Tetanus Toxin/Toxoid IgG ELISA kit, 96 tests, Quantitative |
| 930-220-TRM | Rabbit Anti-Tetanus Toxin/Toxoid IgM ELISA kit, 96 tests, Quantitative |
| 930-310-TGG | G. pig Anti-Tetanus Toxin/Toxoid IgG ELISA kit, 96 tests, Quantitative |
| 930-320-TGM | G. pig Anti-Tetanus Toxin/Toxoid IgM ELISA kit, 96 tests, Quantitative |
| 930-410-TKG | Monkey Anti-Tetanus Toxin/Toxoid IgG ELISA kit, 96 tests, Quantitative |
| 930-500-HTG | Horse Anti-Tetanus Toxin/Toxoid IgG ELISA kit, 96 tests, Quantitative |
| 930-510-HFA | Horse Anti-Tetanus Toxin/Toxoid IgG-Fab2 ELISA kit, 96 tests, Quantitative |
| 940-100-DHG | Human Anti-Diphtheria Toxin/Toxoid IgG ELISA kit, 96 tests, Quantitative |
| 940-110-DHM | Human Anti-Diphtheria Toxin/Toxoid IgM ELISA kit, 96 tests, Quantitative |
| 940-120-DMG | Mouse Anti-Diphtheria Toxin/Toxoid IgG ELISA kit, 96 tests, Quantitative |
| 940-125-DMM | Mouse Anti-Diphtheria Toxin/Toxoid IgM ELISA kit, 96 tests, Quantitative |
| 940-130-DRG | Rabbit Anti-Diphtheria Toxin/Toxoid IgG ELISA kit, 96 tests, Quantitative |
| 940-135-DRM | Rabbit Anti-Diphtheria Toxin/Toxoid IgM ELISA kit, 96 tests, Quantitative |
| 940-140-DGG | Guinea Pig Anti-Diphtheria Toxin/Toxoid IgG ELISA kit, 96 tests, Quantitative |
| 940-145-DGM | Guinea Pig Anti-Diphtheria Toxin/Toxoid IgM ELISA kit, 96 tests, Quantitative |
| 940-150-HFA | Horse Anti-Diphtheria Toxin/Toxoid IgG (Fab2) ELISA kit, 96 tests, Quantitative |
| 940-200-DHG | Human Anti-CRM197 (Diphtheria Toxin mutant) IgG ELISA kit, 96 tests, |
| 940-210-DHM | Human Anti-CRM197 (Diphtheria Toxin mutant) IgM ELISA kit, 96 tests, |
| 940-220-DMG | Mouse Anti-CRM197 (Diphtheria Toxin mutant) IgG ELISA kit, 96 tests, |
| 940-225-DMM | Mouse Anti-CRM197 (Diphtheria Toxin mutant) IgM ELISA kit, 96 tests, |
| 940-230-DRG | Rabbit Anti-CRM197 (Diphtheria Toxin mutant) IgG ELISA kit, 96 tests, |
| 940-235-DRM | Rabbit Anti-CRM197 (Diphtheria Toxin mutant) IgM ELISA kit, 96 tests, |
| 940-240-DKG | Monkey Anti-Diphtheria Toxin/Toxoid IgG ELISA kit, 96 tests, Quantitative |
| 940-245-DKM | Monkey Anti-Diphtheria Toxin/Toxoid IgM ELISA kit, 96 tests, Quantitative |
| 960-110-PHG | Human Anti-B. pertussis antigens (Pertussis toxin, FHA and LPS) IgG, 96 tests, |
| 960-120-PHG | Mouse Anti-B. pertussis antigens (Pertussis toxin, FHA and LPS) IgG ELISA kit, |
| 960-130-PMG | Mouse Anti-B. pertussis toxin/toxoid IgG ELISA kit, 96 tests, Quantitative |
| 960-140-PMM | Mouse Anti-B. pertussis toxin/toxoid IgM ELISA kit, 96 tests, Quantitative |
| 960-150-PRG | Rabbit Anti-B. pertussis toxin/toxoid IgG ELISA kit, 96 tests, Quantitative |
| 960-160-PRM | Rabbit Anti-B. pertussis toxin/toxoid IgM ELISA kit, 96 tests, Quantitative |
| 960-200-PHA | Human Anti-B. pertussis antigens (Pertussis toxin, FHA and LPS) IgA ELISA kit, |
| 960-220-PHM | Human Anti-B. pertussis antigens (Pertussis toxin, FHA and LPS) IgM ELISA kit, |
| 960-225-PHM | Monkey Anti-B. pertussis antigens (Pertussis toxin, FHA and LPS) IgM ELISA kit, |
| 960-230-PGG | Mouse Anti-B. pertussis Pertactin IgG ELISA kit, 96 tests |
| 960-240-PRG | Rabbit Anti-B. pertussis Pertactin IgG ELISA kit, 96 tests |
| 960-250-PHG | Human Anti-B. pertussis Pertactin IgG ELISA kit, 96 tests |

Instruction Manual No. M-VAC-DTX-50

VacciGel Direct ELISA for the measurement of Diphtheria Toxoid (DTX) formulated in Alum (Adjuphos)

Cat. #. VAC-DTX-50; 50 Tests

For In Vitro Research Use Only



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DRAFT MANUAL: PLEASE CONSULT THE MANUAL SUPPLIED WITH THE KIT FOR ANY LOT SPECIFIC CHANGES.

Vaccigel Diphtheria Toxoid (DTX) ELISA KIT #VAC-DTX-50, 50 tests

| Kit Components, 50 tests | Cat # |
|---|------------|
| Vaccigel DTX Std. A (0 mLf/ml), 0.65 ml | VACDTX-51A |
| Vaccigel DTX Std. B (187.00 mLf/ml), 0.65 ml | VACDTX-51B |
| Vaccigel DTX Std. C (375 mLf /ml), 0.65 ml | VACDTX-51C |
| Vaccigel DTX Std. D (750 mLf /ml), 0.65 ml | VACDTX-51D |
| Vaccigel DTX Std. E (1500 mLf /ml), 0.65 ml | VACDTX-51E |
| Vaccigel DTX Std. F (3000 mLf /ml), 0.65 ml | VACDTX-51F |
| Note: All Stds are Alhydrogel/Adjuphos suspension. Must shake well and mix before use as the gel may settle quickly. Stds concn may be lot specific and should be used for actual calculation. | |
| Anti-DTX-HRP Conjugate, 1.0 ml (10X), Dilute 1:10 with HRP Conj. Diluent | VACDTX-52 |
| HRP Conjugate Diluent, 10 ml | SD20T-1 |
| Vaccigel Sample Diluent, pink solution, 15 ml (mix the contents prior to use) | VACDTX-53 |
| LowNSB Diluent (Green solution), 15 ml (mix the contents prior to use) | TBTm |
| Wash buffer (100X), 10 ml; dilute 1:100 with water | WB-100 |
| HRP substrate, Solution, 12 ml | 80091 |
| Stop solution, 12 ml | 80101 |
| ELISA Strip Plate (8x12 or 96 wells) | VAC-P1 |
| Vaccigel Assay Tubes, 50 | VACT-50 |
| Vaccigel Qtips, 50 | VACQ-50 |
| Instruction Manual | VAC-DTX-50 |

A vaccine is a biological preparation that improves immunity to a particular disease. Some vaccines also contain chemicals called adjuvants to help stimulate the production of immunity against the vaccine active ingredients, making the vaccine more effective. Currently, the only adjuvants approved for human vaccine are aluminum containing compounds, including aluminum hydroxide or Alhydrogel®, aluminum phosphate, and potassium aluminum sulfate or alum. Aluminium adjuvants have been used in tetanus, diphtheria, pertussis, polio, rabies, and hepatitis A and B vaccines. To ensure vaccine quality, regulatory authorities require the manufacturer to measure vaccine content in the final product. World Health Organization (WHO) recommends that at least 80% of the vaccine be adsorbed to the gel. In particular, it is essential to determine the amount as well as the identity and integrity of the antigens bound to aluminum containing adjuvants following formulation. Aluminum-based gels are typically fibrous or beaded in suspension. The presence of aggregates, turbidity, flocculent gels or beads in solution prevents direct quantitation of protein content in formulations using protein assays such as Lowry, BCA, or Bradford protein assay, not to mention that these assays are all non-specific and low in sensitivity. Alhydrogel formulations also do not allow complete dissolution or extraction making it very difficult to know the identity of the vaccines or know the amount of the protein after their dispensing. There have been several incidents of mislabeling of anti-fertility vaccine with tetanus vaccines. Therefore, there is an urgent need for a test not only to identify but measure the vaccine contents.

The Vaccigel™ ELISA for Diphtheria Toxoid (DTX) is the first commercial test to measure the active component of DTX vaccines formulated in Adjuphos (Alum gel). It is a simple, rapid, and sensitive test and required no extraction or harsh dissociation of the antigens from the gel (Alum). This kit has been validated with Daptacel (DTAP, Diphtheria and Tetanus Toxoids and Acellular Pertussis Vaccine Adsorbe, Sanofi). This kit can be used to measure DTX in monovalent of multivalent vaccines adsorbed on Alum (Trihibit (DTAP/Hib), ActHib (Hib-PRP-T), Trihibit (DTAP/Hib), Daptacel (DTAP), Tripedia (DTAP), Td (Adult), Decavac™ (tetanus/Diphtheria), Adacel (tetanus, Diphtheria, Acellular Pertussis), DT (Pediatric) - Sanofi Pasteur; Pediarix (DTAP/HepB/IPV), Infanrix (DTAP), Boostrix (Tetanus, Diphtheria, Acellular Pertussis)- GlaxoSmithKline). ADI has another kit for measuring free DTX in biological buffer (#VAC-DTX-210)

QUALITY CONTROL

Standards and controls must perform as stated in the manual. This kit is tested, optimized, and calibrated with DTX vaccine (DTap Sanofi). This vaccine or other approved vaccines can be used as external control.

PERFORMANCE CHARACTERISTICS

DETECTION LIMIT- Based on replicate determinations of the zero standard, the minimum DTX vaccine concentration detectable using this assay is ~75 mLf/ml. The detection limit is defined as the value deviating by 2 SD from the zero standard.

Specificity

Vaccigel Direct DTX ELISA has been tested and calibrated with FDA-approved DTX vaccine DTap (Daptacel, Sanofi, Triple vaccine containing TTX, Diphtheria Toxoid, and Pertussis antigens). This kit can be used to measure Tetanus Toxoid in monovalent of multivalent vaccines adsorbed on Alum (Trihibit (DTAP/Hib), ActHib (Hib-PRP-T), Trihibit (DTAP/Hib), Daptacel (DTAP), Tripedia (DTAP), Td (Adult), Decavac™ (tetanus/Diphtheria), Adacel (tetanus, Diphtheria, Acellular Pertussis), DT (Pediatric) - Sanofi Pasteur; Pediarix (DTAP/HepB/IPV), Infanrix (DTAP), Boostrix (Tetanus, Diphtheria, Acellular Pertussis)- GlaxoSmithKline). ADI has another kit for measuring free tetanus toxoid in biological buffer (#VAC-DTX-310).

This kit is not suitable to measure DTX protein in solution or on non-alum formulations of the vaccines. ADI has developed other ELISA kits for the measurement of both DTX or the antibodies. This kit has not been validated to measure active DTX (non-toxoid, or its subunits).

Suggestions for good performance for Vaccigel ELISA

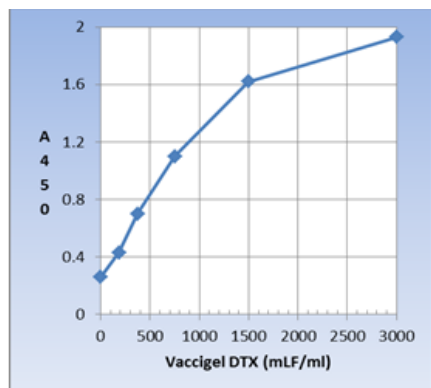
Vaccigel ELISA is an unusual test because of antigen-antibody reaction is being performed directly on the vaccine samples that are in gel suspension or precipitate. Therefore, it is very important to take a uniform amount of the sample gel and dilute it in Vaccigel diluent (pink) in order to avoid loss of the small gel particle during prolong processing of the test. You must get a good understanding of the protocol and the role of each steps. The Vaccigel ELISA differs from the regular ELISA due to the nature of the samples (particulate or Alum gels). It is most critical to be patient during the manual wash process and remove traces of the wash solutions and **without losing the sample** (gel pellet). Some common issues:

- High standards give low values-** Generally due to the loss of the standard/sample gel pellet during the assay; not mixing the standards before taking the samples; Stds/sample not diluted in Vaccigel diluent; Antibody-HRP used at lower concn or higher dilution than the recommended 1:10.
- Standards or duplicates show high variations-** One or more of the issues as stated in item #1.
- Vaccine gel pellet too tight and does not resuspend easily –** It is due to higher speed or time. We recommend centrifuging assay tubes at 3000 rpm, 30 secs at each step. Lower the speed or time to correct the problem.
- Vaccine gel pellet too lose and risk of loss –** It is due to lower speed or time. We recommend 3000 rpm, 30 secs at each step. Adjust speed or time.
- It is a good idea to run just the standards and a few samples to get familiar with the protocol before running too many samples to avoid unusual delays at various steps.

WORKSHEET OF TYPICAL ASSAY

| Wells | Stds/samples | |
|--------|------------------------------------|------|
| A1, A2 | Vaccigel DTX Std. A (0 mLF/ml) | 0.26 |
| B1, B2 | Vaccigel DTX Std. B (187 mLF /ml) | 0.43 |
| C1, C2 | Vaccigel DTX Std. C (375 mLF /ml) | 0.7 |
| D1, D2 | Vaccigel DTX Std. D (750 mLF /ml) | 1.1 |
| E1, E2 | Vaccigel DTX Std. E (1500 mLF /ml) | 1.62 |
| F1, F2 | Vaccigel DTX Std. F (3000 mLF /ml) | 1.93 |
| F1, F2 | Sample 1 at 1:100 | 1.06 |

NOTE: These data are for demonstration purpose only. A complete standard curve must be run in every assay to determine sample values. Each laboratory should determine their own normal reference values.



/1_ADL_ELSA_

A typical std. assay curve (do not use this for calculating sample values)

CALCULATION OF RESULTS

Calculate the mean absorbance for each duplicate. Subtract the absorbance of the zero standard from the mean absorbance values of standards, control, and samples. Draw the standard curve on a graph paper by plotting net absorbance values of standards against appropriate DTX concentrations. Read off the DTX concentrations of the control and samples directly from the standard curve. If samples were diluted then the values should be multiplied by the dilution factor.

If ELISA reader software is being used, we recommend 4-parameter or 5-parameter curve.

Vaccigel Std Calibration

DTX vaccine Standards and controls are calibrated with FDA-approved DTX vaccine formulated in Alum/Alhydrogel (Dtap or Daptacel, Sanofi; 3,000 mLF/ml). Vaccines from other manufacturers may give a different concentration due to the recombinant protein concn or the formulations of Alum or % of Aluminum. Therefore, we recommend that an internal reference is always used.

Sample Dilution and recovery

DTX Vaccine samples (Dtap) when diluted 1:50, 1:100, 1:200, and 1:400 in Vaccigel diluent (pink solution) showed good recoveries.

PRINCIPLE OF THE TEST

Vaccigel DTX ELISA kit is based on direct binding of anti-DTX antibody-HRP conjugate to DTX adsorbed on the gel. After a washing step, chromogenic substrate is added and colors developed. The enzymatic reaction (color) is directly proportional to the amount of DTX present in the sample. Adding stopping solution terminates the reaction. Absorbance is then measured on a microtiter well ELISA reader at 450 nm. and the concentration of DTX in samples and control is read off the standard curve.

MATERIALS AND EQUIPMENT REQUIRED

Adjustable micropipet (100-1000 ul) and multichannel pipet with disposable plastic tips. Reagent troughs, plate washer (recommended) and ELISA plates Reader. Table top microfuge

PRECAUTIONS AND SAFETY INSTRUCTIONS

ADI Vaccigel ELISA kit is intended for *in vitro research* use only. The reagents contain proclin-300 (0.1%) as preservative; necessary care should be taken when disposing solutions. Applicable MSDS, if not already on file, for the following reagents can be obtained from ADI or the web site. TMB (substrate), H₂SO₄ (stop solution), and Proclin-300 (0.1% v/v in standards, sample diluent and HRP-conjugates).

http://4adi.com/commerce/info/showpage.jsp?page_id=1060&category_id=2430&visit=10

SAMPLE COLLECTION AND HANDLING

This kit is designed to measure the DTX in vaccine formulated in Alum (Adjuphos). **Do not add azide** or other preservatives that may inactivate the enzyme. **Do not freeze the vaccines.** This kit is not suitable to measure DTX in serum or in solution (without Alum).

REAGENTS PREPARATION FOR THE ASSAY

Dilute wash buffer (1:100) with distilled water (10 ml stock in 990 ml). Store at 4oC.

Sample Dilutions. Note the concentration of DTX in the vaccine (example, DTX vaccine DTap by Sanofi is supplied at 30 Lf/ml or 3,000 mLF/ml of the suspension). It should be diluted 1:50-200 or more for testing. We suggest to dilute the vaccine in 2-steps: Make an initial 1:10 stock of vaccine samples (e.g., 20 ul of vaccine and 180-ul of the antibody conjugate/sample diluent. This stock should be used to prepare further test dilutions of 1:100 or higher in Vaccine diluents (pink solution; 25 ul of 1:10 stock and 225-ul of vaccine diluent for a final dilution of 1:100). Unused 1:10 stocks of the vaccine samples should be stored at 1:10 stock as the diluent has protein additives and preservatives. Diluted stock are stable in this diluent for up to 4-6 weeks. Last dilution of all samples must be done in the supplied Vaccigel diluent (pink solution) only. Do not use any other diluent. For unknown vaccine samples, prepare initial dilutions in Antibody diluent and final test dilution in Vaccigel diluent. All samples must be tested in duplicate.

Dilute antibody-enzyme conjugate 1:10 (eg; 100 ul of HRP-conjugate in 900 ul HRP conjugate diluent). Prepare 1.5 ml for 10 samples or prepare as needed; 150 ul of diluted conjugate per sample). Do not keep working stock of diluent beyond the assay date. Prepare only in required amounts.

Note: Do not use HRP/antibody diluent from another lot of the same kit or different kits. This is a very specific diluent for diluting anti-DTX-HRP conjugate.

STORAGE AND STABILITY

The kit contents, if unopened, are stable at 2-8°C until the expiration date printed on the label. The whole kit stability is at least 6 months from the date of shipping under appropriate storage conditions.

TEST PROCEDURE (ALLOW ALL REAGENTS TO REACH ROOM TEMPERATURE BEFORE USE).

All standards, controls, and samples should be tested in duplicate. Remove required number of supplied 1.5 ml assay tubes, corresponding # strips and arrange them on the plate. Store unused tubes and strips in the supplied bag. **Dilute wash buffer 1:100** with water. **Dilute HRP conjugate 1:10** in HRP Conjugate diluent. Multivalent vaccines such DTAP (diphtheria, tetanus, pertussis) are supplied in Alhydrogel or Adjuvophos (Alum gels). It is a gel suspension. Gently mix the vaccine gel suspension by inverting the contents a few times and then gently mixing it for 5-10 mins at room temp. The vaccine suspension, if not mixed and allowed to sit, will settle at the bottom. Therefore, take the samples for testing immediately after mixing. Dilute vaccine samples in the supplied Vaccigel diluent only (Pink solution).

1. Label the required # of 1.5 ml assay tubes and label the required # of blank ELISA strips as well. Do not waste the assay tubes or the blank strip wells. If necessary break off the wells and arrange them on the well holder. The ELISA wells are only used to read the A450 values of the samples at the end of the assay. It is possible to use strips or ELISA plates from other suppliers as well.
2. **Dilute Vaccine samples** formulated in Alhydrogel **1:100-200** (or as necessary;) in Vaccigel diluent only (pink solution). Dtap vaccine, for example has TTX at 10,000 mLF/ml in Adjuvophos so 1:100 dilution will be ~100 mLF/ml and within the testing range of the assay. The gel will settle at the bottom during storage. It should be gently mixed for 5-10 seconds before use. Please review sample dilution scheme on page 2.
3. Low NSB diluent is a Vaccigel stabilizer solution (green). It is a turbid or have cloudy appearance. It should be gently mix by manual shaking or inverting the bottle for 5-10 seconds prior to every use. **Pipet 300 ul solution** to appropriate # of labeled 1.5 ml conical assay tubes supplied in the kit.
4. **Do not dilute standards.** Gently mix the standards by vortexing for 5-10 seconds. **Dispense 100 ul of the standards, vaccine samples** (diluted) in duplicate into the tubes containing **300-ul green diluent. Close the caps and Gently mix the contents by vortexing for 2-3 seconds; incubate for 30 mins at room temp.**
5. Centrifuge the tubes for 30 seconds in a microfuge at 3000 rpm at room temp. **Note** the pinkish/brownish small pellet of Alum gel at the bottom of all tubes. Carefully invert the tube and discard the entire content in waste container. Keep the tubes inverted and tap over the paper towels a few times to remove the remaining solution.

You must not disturb the gel pellet or discard it as it contains the vaccine active ingredients. The pellet will remain at the bottom of the tube during the process. Return all tubes to the tube holder. This process must be done 1 tube at a time and maintain the proper sequence of the tubes.

6. Add **150 ul of diluted (1:10) antibody-HRP conjugate.** Vortex each tube 3-5 seconds to mix the pellet with the conjugate solution. **Note:** The gel pellet must have a uniform suspension, failing which you will get lower reading or high variance in duplicates.. After mixing, **incubate all tubes for 60 min** at RT.
7. Centrifuge the tubes for 30 seconds in a microfuge at 3000 rpm at room temp as in step 5. **Note** the small pellet of Alum gel at the bottom of each tube. Carefully invert the tube and remove the conjugate solution as in step 5. Wash the pellet by adding **300 ul** 1x wash buffer into all tubes. Vortex to mix and resuspend the pellet to make uniform suspension. Repeat the pellet wash 4-times more for a total of 5-washed. **Note:** After each wash, the tubes must be tapped over the paper towels to remove the liquid. Failure to wash properly will produce higher blanks.
8. After the last wash, remove all liquid from the tube or the walls and tap over fresh the paper towels. Observe each tube for any liquid or droplets sticking on the tube walls. Remove traces of conjugate from the tube walls using supplied Qtips. **Note:** failure to remove the wash solution will result into higher blanks. Do not disturb the pellet or touch with the Qtip.
9. **Dispense 150 ul TMB substrate solution per tube.** Close the cap and vortex each tube 3-5 seconds to make sure that the gel pellet is completely dispersed. Failure to perform this step properly will give spurious reading or irregular duplicates. **Incubate for 15 minutes at room temp.** Note: Incubation time may be changed ± 5 min so as to get maximum A450 =2.00-3.00). Blue color develops in standards and positive tubes.
10. **Stop the reaction by adding 150 ul of stop solution** to all tubes. Mix gently for 3-5 seconds to ensure even color distribution. Blue color turns yellow.
11. **Centrifuge the tubes for 30-seconds at 3000 rpm. Carefully take 200 ul of the supernatant (yellow) using a pipette and transfer to the ELISA strip wells for reading.** The order of the wells should be the same as for the tubes.
12. Measure the absorbance at 450 nm using an ELISA reader. Color is stable for at least 30 mins after stopping.

