

Neonatal MSUD Screening Assay

Born Safe
MSUD

TM



Enzymatic assay for the detection of Maple Syrup Urine Disease (MSUD) in new born dried blood spots

(FOR IN VITRO DIAGNOSTIC USE ONLY)

1. INTENDED USE

Born Safe™ Neonatal MSUD Screening Assay is an enzymatic colorimetric method for the quantitative detection of Maple Syrup Urine Disease in new born using blood spot samples dried on Whatman S&S 903 filter collection paper. This test is intended as a screening method for measuring the L-branched-chain amino acid (BCAA) concentrations in newborn blood spot specimens. Elevated results are not diagnostic per se of maple syrup urine disease, but indicate the urgent need for further study of the newborn from which the presumptive positive specimen was received. The kit should not be used for confirmatory testing or to monitor therapy

2. SUMMARY AND EXPLANATION OF THE ASSAY

Maple Syrup Urine Disease (MSUD), also known as branched-chain ketoaciduria, is due to a deficiency of the branched-chain ketoacid decarboxylase enzymes.¹¹ The disease is characterized by elevated circulating analyte concentrations, namely L-branched-chain amino acids. Three essential amino acids-leucine, isoleucine and valine-are often called the branched-chain amino acids (BCAAs). Because of the enzyme deficiency in MSUD, the BCAAs and their by-products, called ketoacids, become elevated. It is these elevations that cause an infant or child with MSUD to become symptomatic.

Four general classifications are used to identify the types of MSUD: classical, intermediate, intermittent and thiamine-responsive forms based on clinical presentation and outcome.¹³ These terms refer to the amount and type of enzyme activity present in the affected child, which can vary considerably within each classification. Each variant is due to deficient decarboxylation of the branched-chain ketoacids and in each case, all three ketoacids and their respective amino acids are increased in the circulation.

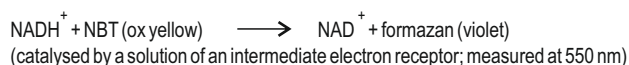
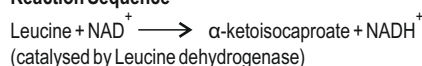
Classical is the most common type of MSUD, leucine concentrations reach abnormally high levels within hours of birth regardless of protein intake.⁹ In classical MSUD, little or no enzyme activity (usually less than 2% of normal) is present. Infants with classical MSUD will show symptoms within the first several days of life. They generally have poor tolerance for the BCAAs, so protein must be severely restricted in their diet. Intermediate MSUD is a variant of the classical type of the disease. Those with intermediate MSUD have a higher level of enzyme activity (approximately 3 to 8% of normal). They can usually tolerate a greater amount of leucine. Intermittent MSUD is a milder form of the disease because of the greater enzyme activity present (approximately 8 to 15% of normal). Often the child does not have symptoms until 12 to 24 months of age, usually in response to an illness or surge in protein intake. During episodes of illness or fasting, the BCAA levels elevate, the characteristic maple syrup (or burnt sugar) odour becomes evident, and the child can go into a metabolic crisis.^{1,2,3,4,5}

Maple Syrup Urine Disease has an incidence of 1 in 225 000 newborns.¹⁰ Increased circulating concentrations of L-BCAAs and α-ketoacids, if untreated, cause severe mental and motor retardation the severity of which can be lessened by prompt dietary restriction of L-BCAAs.¹² However, delayed treatment leads to these chronic symptoms becoming irreversible.

3. PRINCIPLE OF THE ASSAY

The Leucine and BCAAs from cellulose paper (dried blood spot samples) are extracted with trichloroacetic acid (Elution buffer). After extraction, the eluted sample is combined with the enzyme reagent Leucine dehydrogenase. This enzyme reagent catalyses the NAD-dependent oxidative deamination of Leucine and L-BCAAs to α-ketoisocaproate acid. The NADH produced, reacts with a colour reagent in which a tetrazolium salt gets reduced producing a distinct colour. This colour can be measured colorimetrically with a photometer at 550 nm and is directly proportional to the concentration of Leucine and BCAA present in the sample.

Reaction Sequence



4. PRESENTATION

| REF | ▽ |
|------------|-----------|
| 1122030096 | 96 Assays |

5. KIT COMPONENTS:

Reagents: (96T Pack size)

- Calibrators and Controls blood spots:** 1+1 set of blood spots cards of human whole blood spotted onto Whatman S&S 903 paper containing 5 calibrators and 2 controls with low and high concentrations of Leucine. Refer to the quality control sheet for the exact concentrations of the Calibrators and acceptable range values of the Controls.

- Elution Buffer:** 1 x 10.0 ml of TCA 3% w/v. Ready to use.
- Enzyme Reagent:** 4 x 1.0 ml of Leucine dehydrogenase lyophilized with buffer and a stabilizer. Reconstitute each vial with 1.0 ml of distilled water. After reconstitution, the reagent can be stored at 2-8°C for one month.
- Coenzyme Reagent:** 4 x 1.0 ml of Lyophilized NAD. Reconstitute each vial with 1.0 ml of distilled water. After reconstitution, the reagent can be stored at 2-8°C for one month.
- Colour Reagent:** 1 x 8.0 ml of tetrazolium salt. Ready to use. Preservative: NaN₃ (<0.1%).
- Colour Booster:** 1 x 1.0 ml of a solution of an intermediate electron receptor in buffer. Ready to use. Preservative NaN₃ (<0.1%).
- Dilution Buffer:** 1 x 2.0 ml of buffer. Ready to use. Preservative NaN₃ (<0.1%).

| Reagents | Quantity | Physical State |
|-------------------------|-------------|-------------------|
| Calibrator and Controls | 1 set each | Dried blood spots |
| Elution Buffer | 1 x 10.0 ml | Ready to use |
| Enzyme Reagent | 4 x 1.0 ml | Lyophilized |
| Co-enzyme Reagent | 4 x 1.0 ml | Lyophilized |
| Colour Reagent | 1 x 8.0 ml | Ready to use |
| Colour Booster | 1 x 1.0 ml | Ready to use |
| Dilution Buffer | 1 x 2.0 ml | Ready to use |

Accessories:

- Round bottom microtiter plates** (Elution Plates).
- Flat-bottom microtiter plates** with superior optical quality (Assay Plates).

6. STORAGE AND STABILITY OF THE KIT

- Store all reagents at 2-8°C when not in use. Calibrators and Controls should be stored protected from moisture and light in the original bag with desiccant. Stable at 2-8°C until expiry date stated on the label. Make sure that the plastic bag remains sealed during storage.
- We recommend that the Blood Spots (Calibrators and Controls) should be preferably stored at -20°C with desiccants when not in use for prolonged periods.**
- Unopened reagents will retain reactivity until expiration date shown on the label. Do not use reagents beyond this date.

7. MATERIALS REQUIRED BUT NOT PROVIDED

- Distilled or de-ionized water.
- Adjustable, automatic micropipettes with disposable tips.
- Microtiter plate reader equipped with 550 nm filter in endpoint reading mode.
To be Procured Separately
- Blood spot puncher 3.2 mm.
- Orbital plate shaker (900 rpm).
- Blood spots collection cards [Whatman Schleicher & Schuell 903 recommended; CLSI NBS01-A6 compliant].

8. WARNINGS AND PRECAUTIONS

A thorough understanding of the pack insert is mandatory before performing the test for the first time. Adherence to the protocol specified herein is necessary to ensure optimal performance of the product. Any deviation from the assay procedure may affect the results.

Operating: In order to obtain reproducible results, the following rules must be observed:

- Do not mix reagents of different lots.
- Do not use reagents beyond their expiry date.
- Use thoroughly clean glassware.
- Use distilled water, stored in clean containers.
- Avoid any contamination among samples; for this purpose, disposable tips should be used for each sample and reagent.
- Keep all reagents at normal refrigerator temperature (2-8°C) in closed containers when not in use, but ensure that all reagents are equilibrated to 18-25°C before use. Keep Blood Spot Standards and Controls at normal refrigerator temperature (2-8°C) in the original foil pouch containing desiccant when not in use, but ensure that spots are equilibrated to 18-25°C before use.

Safety: In order to avoid personal and environmental contamination, the following precautions must be observed:

- Use disposable gloves while handling potentially infectious material and performing the assay.
- Do not pipette reagents by mouth.
- Do not smoke, eat, drink or apply cosmetics during the assay.
- All material of human origin used for the preparation of this kit tested negative for HBsAg, anti-HIV and anti-HCV. Since no test at present can guarantee complete absence of these viruses, all samples and reagents used for the assay must be considered potentially infectious. Therefore, the assay waste must be decontaminated and disposed of, in accordance with established safety procedures.
- Disposable ignitable material must be incinerated; disposable non-ignitable

material must be sterilized in autoclave for at least 1 hour at 121°C. Liquid wastes must be added with sodium hypochlorite at a final concentration of 3%. Let the hypochlorite act for at least 30 minutes. Liquid wastes containing acid must be neutralized with appropriate amounts of base, before treating with sodium hypochlorite.

6. Avoid splashing and aerosol formation; in case of spillage, wash carefully with a 3% sodium hypochlorite solution and dispose of this cleaning liquid as potentially infectious waste.
7. Some reagents contain sodium azide as preservative; to prevent build-up of explosive metal azides in lead and copper plumbing, reagents should be discarded by flushing the drain with large amounts of water.
8. **Caution:** Elution Buffer containing trichloroacetic acid (TCA), is highly acidic and corrosive. Protective gloves and safety glasses should be worn while using this reagent.

9. SPECIMEN COLLECTION AND HANDLING

Blood samples should ideally be collected between the third and the fifth day of life (48 to 120 hours after birth) and should be taken directly from a heel prick onto filter paper. Neonatal screening programs differ from one another in the type of specimen required, the recommendation is a blood spot, approximately 12.7 mm (½ inch) in diameter, collected by heel prick and spotted onto filter paper (Whatman Schleicher & Schuell 903). The specimen collection device must comply with national regulations. A method based on dried blood samples requires skilful collecting, handling and transport of samples. The collection technique is described in detail in CLSI document LA4-A5,⁹ and the main points are listed below.

- Blood from the new-born's heel should be collected **ONLY** from the medial (closest to the body center-line) or lateral portion (furthest from the body center-line) portion of the planter surface (walking surface).
- Blood collection from other areas of the infant's foot, e.g. arch, may result in nerve, tendon or cartilage injury.
- Clean the skin with an alcohol swab and allow to air-dry.
- Puncture the infant's heel with a sterile lancet or with a heel incision device to the depth of approximately 2.0 mm. Puncturing deeper than 2.0 mm on small infants may cause bone damage.
- Wipe away the first drop of blood. Gently touch the filter paper against a large drop of blood and, in one step, allow a sufficient quantity of blood to soak through to completely fill a pre-printed circle on the filter paper. Examine both sides of the filter paper to make sure that the blood penetrated and saturated the paper. Excessive milking or squeezing the puncture may cause haemolysis of the specimen or an admixture of tissue fluids with the specimen. Do not layer successive drops of blood in the collection circle (this causes caking).
- Allow the blood specimen to air-dry in a horizontal position for at least 4 hours at ambient temperature (18-25°C). Do not heat or stack the specimens during the drying process.
- Arrange transport of the collection card to the screening laboratory within 24 hours of collection. L-BCAAs in dried blood spot specimens have been shown to be very stable, under a variety of storage conditions. However, it is recommended that a repeat specimen is requested if a period of 10 days or more has elapsed between collection and sample testing. Additionally, the conditions of the collection card storage must be consistent with the stability of the least stable analyte to be measured in the specimen.
- Store in sealed paper envelopes or containers that will provide protection from moisture, light, heat and contact with other materials.
- The sample discs should be punched from similar areas on each individual blood spot. Do not punch sample discs from areas that include printed marks or that are near the edges of the blood spot.
- Be sure that the required information on the specimen collection card has been completed. The minimum pre-printed information required on the collection device includes:
 - ◆ last name (and first, if available), sex, birth date (optional: time of birth), birth weight and age of the infant; (indicate if < 24 h), and patient identification number
 - ◆ the first and last name of the mother
 - ◆ date of specimen collection (optional: time of collection)
 - ◆ the name and address of the submitter (optional: birth facility)
 - ◆ the name and phone number of the physician (health care provider)
 - ◆ the name of the new born screening program and address
 - ◆ each card should have a unique serial number and an expiration date.
- Specimens should not be placed in hermetically sealed containers (e.g. plastic or foil bags). If required, sufficient desiccant packages must be included. Humidity and moisture are detrimental to the dried blood spot specimen.
- Before placing the specimens in a container for transport, the dried blood spots on the collection cards should be separated by a physical barrier from the blood spots on the cards in the stack immediately above and below. The blood spots can also be protected by a fold-over cover attachment or by placing glassine paper between the specimens.

Note: Transport the specimen to the laboratory within 24 hours after collection.

10. REAGENTS PREPARATION ENZYME-COENZYME SOLUTION

- A. **Reconstitution:** First reconstitute, one Enzyme vial and one Coenzyme

vial with 1 ml of distilled or de-ionized water each. Mix gently to aide reconstitution. After reconstitution, the reagent can be stored at 2-8°C for one month.

B. Preparation:

Mix 2 parts of Enzyme reagent with 2 parts of Coenzyme reagent and 1 part of Dilution buffer.

The following table gives the volumes required for each of the three components to run specific number of tests (volumes in µl).

| No. of tests | Enzyme (µl) | Co-enzyme Reagent (µl) | Dilution Buffer(µl) | Enzyme- Co-enzyme solution. Total vol.(µl) |
|--------------|-------------|------------------------|---------------------|--|
| 10 | 400 | 400 | 200 | 1000 |
| 20 | 800 | 800 | 400 | 2000 |
| 40 | 1600 | 1600 | 800 | 4000 |
| 80 | 3200 | 3200 | 1600 | 8000 |
| 100 | 4000 | 4000 | 2000 | 10000 |

We highly recommend the addition of the Dilution buffer just before using the mixture. Do not keep or use the reconstituted Enzyme, Coenzyme, or the combined Enzyme-Coenzyme working solution for any longer than the specified periods of time.

COLOR REAGENT MIXTURE

Prepare the mixture by adding 1 part of Colour Booster to 10 parts of Colour reagent.

| No of Tests | Colour Booster (µl) | Colour Reagent (µl) | Colour reagent mixture Total volume (µl) |
|-------------|---------------------|---------------------|--|
| 10 | 80 | 800 | 880 |
| 20 | 160 | 1600 | 1760 |
| 40 | 320 | 3200 | 3520 |
| 80 | 640 | 6400 | 7040 |
| 100 | 800 | 8000 | 8800 |

After reconstitution keep the Colour Reagent mixture away from the direct light (i.e. wrapped in aluminium foil); stable for 4 hours at 2-8°C. Not to be left out of the refrigerator longer than needed. Take the colour reagent out of the refrigerator just prior to use. Take out just the quantity you are going to use for the day. Return the rest of the colour reagent in the refrigerator.

11. ASSAY PROCEDURE

A. ELUTION STEP:

1. Bring all reagents (except the colour reagent) to room temperature before pipetting.
2. Punch 2 spots of C0 for blank and 2 blood spots of **Calibrators (C1-C5), Controls (L1, L2)** and **Samples** (each 3.2 mm diameter) Put 2 discs into the respective wells of the round bottom microtiter plate.
3. Pipette **100 µl** of **Elution Buffer** into each well. Ensure that each disk is fully immersed in the liquid.
4. Incubate the microtiter plate on an orbital plate shaker (900 rpm) for **30 minutes** at room temperature (**20-26°C**).
5. During the elution, reconstitute and prepare the reagents (section 10), and a flat bottom microtitre pipette.

B. SAMPLE TRANSFER AND ASSAY:

6. After the incubation, remove the plate from the plate shaker and transfer 40 µl of the eluate from each well to the corresponding wells of the flat bottom microtiter plate
7. Pipette **100 µl** of the **Enzyme-Coenzyme** solution prepared in section 10 to each well. Mix well, avoiding the formation of foam.
8. Incubate **30 minutes** at room temperature (20-26°C).
9. Add **80 µl** of **Colour Reagent** mixture prepared in section 10 to each well. Mix well to avoid the formation of foam.
10. After **10 minutes** of incubation at room temperature keeping the plate away from light, measure the microplate at 550-570 nm (optimal: 550 nm), endpoint mode, single measurement. There is no need to wait longer than 10 minutes.

Please note the following:

- This assay is to be performed at room temperature (20-26°C). At higher temperatures (over 28°C) an abnormally high blank may be observed.
- A high blank may also be observed if the colour reagent stage is prolonged more than 20 minutes.

12. CALCULATION OF RESULTS

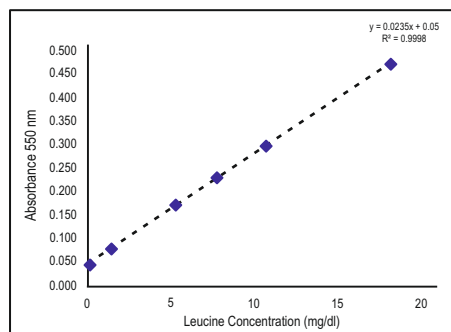
Draw a calibration curve, by plotting the calibrators concentration (x-axis) against the absorbance OD obtained for each calibrator (y-axis). The obtained OD of the standards are plotted against their concentration. The standard curve is calculated by a linear regression function. Using computer programs, the curve is best described by a 2-point linear regression fit with linear axes. Corresponding Leucine concentrations in mg/dL are obtained by interpolating the absorbances of each sample on the calibration curve. Unit conversion: 1mg/dl= 76.3µmol/L.

Example 1

The data presented in Example 1 and Figure 1 are for illustration only and should not be used in lieu of a Calibration graph prepared with each assay run.

| Calibrator | Concentration (mg/dl) | Absorbance |
|------------|-----------------------|------------|
| C0 | 0 | 0.046 |
| C1 | 1.3 | 0.083 |
| C2 | 5.2 | 0.175 |
| C3 | 7.7 | 0.231 |
| C4 | 10.6 | 0.300 |
| C5 | 18.1 | 0.474 |

Figure 1



13. EXPECTED VALUES AND INTERPRETATION CRITERIA

Specific cut-off and reference range should be established by each laboratory with their local demographic population.

The determination of presumptive positives for MSUD is based on the use of a cut-off value which distinguishes between presumptive negative and presumptive positive results. Test results may vary based on infant age at the time the blood is drawn as well as other conditions. Please note that the values given in this section should only be used as a guideline, and it is strongly recommended that each laboratory determine its own reference range and cut-off based on specimens from the laboratory routine population and also a procedure for the follow up of newborns from which a 'presumptive positive' specimen was received. Caution must be exercised in correlating the laboratory result to clinical status with specimens from new-born less than 48 hours after birth, premature and low birth weight new-borns and hospitalized sick newborns. Any sample classified as presumptive positive based on the cutoff should be subjected to further confirmatory testing on the original specimen and a fresh specimen should be performed.

Based on the review of various NBS programs, following guidelines are suggested.

Suggested Cut off Values

| Interpretation | Concentration (mg/dl) |
|----------------------|-----------------------|
| Presumptive Negative | <4.5 mg/dl |
| Presumptive Positive | >4.5 mg/dl |

The mean L-Leucine concentration measured by **Born Safe™** Neonatal MSUD Screening Assay in >300 routine New-born blood spots specimen was 2.35 mg/dl (SD 0.54).

A suggested Cut-off was statistically derived as 4.5 mg/dl; specimens with an L-BCAA concentration of greater than or equal to 4.5 mg/dL would be classified as 'presumptive positive' and a confirmatory test on the original specimen and a fresh specimen should be performed.

14. QUALITY CONTROL

Internal controls (L1-L2) included in the kit should be routinely monitored to check that measured concentrations are within the stated values. These controls provide valuable information regarding the validity of the test according to manufacturer. The assay run is acceptable if the mean concentration for each Control is within the range quoted by the manufacturer for each Control. The assay is unacceptable if values for either of the Blood Spot Controls fall out with the specifications and patient sample results should not be reported. An investigation into the reasons for the assay failure must be undertaken immediately.

If the precision of the assay does not correlate with this standard and repetition excludes errors in technique, check the pipetting and timing devices, instrument calibration, expiration dates on reagent labels and prepared working solutions, storage conditions and temperature control devices. Each laboratory should initiate and document appropriate quality assurance control systems for monitoring and sustaining the accuracy of test results. Users may wish to include further in-house controls and/or Reference materials if available, in addition to Controls L1 and L2. The stability and storage conditions of these additional controls and the criteria for assay acceptance/rejection should be determined by each user laboratory.

The acceptable (mg/dL) Control limits can be displayed graphically e.g. Levey-Jennings or Shewart control charts. The Control limits will represent decision levels for acceptance or rejection of analytical results. Separate charts should be prepared for each Control.

Participation in External Quality Control programs e.g. CDC Infant Screening Performance Surveillance Program is also recommended.

15. ANALYTICAL PERFORMANCE CHARACTERISTICS

Precision: The intra assay and inter assay precision were determined as per NCCLS Evaluation Protocol (EP5-A2).

Within run Precision was determined on 1 Dried blood spot Control and 1 normal sample running in 10 and 20 replicates respectively.

Between run precision was determined on 2 normal samples running in 5 replicates each in four different runs.

Intra and Inter assay Precision:

| Specimen | N | Mean (mg/dl) | SD | Intra Assay Variation (CV%) |
|----------|----|--------------|------|-----------------------------|
| Control | 10 | 15.2 | 0.75 | 4.96 |
| Sample 1 | 20 | 3.17 | 0.28 | 8.97 |
| Specimen | N | Mean (mg/dl) | SD | Inter Assay Variation (CV%) |
| Sample 2 | 20 | 3.24 | 0.32 | 9.97 |
| Sample 3 | 20 | 2.9 | 0.30 | 10.32 |

Precision/Accuracy study using CDC-PT material:

| Sample | N | Target Value (mg/dl) | Mean (mg/dl) | SD | Inter-Assay Variation (CV%) |
|----------------|---|----------------------|--------------|------|-----------------------------|
| Blind Sample 1 | 8 | 8.12 | 8.17 | 0.43 | 5.32 |
| Blind Sample 2 | 8 | 2.46 | 2.95 | 0.26 | 8.80 |

Analytical Sensitivity:

The Assay Linearity & Analytical Sensitivity was assessed as per NCCLS Proposed Guideline EP6-P; by the mean concentration of low levels samples (n=100) which can be statistically distinguished from '0' mg/dl. The Analytical Sensitivity of **BornSafe™** Neonatal MSUD Screening Assay is 0.94 mg/dl.

Interference:

The assay specificity of **BornSafe™** Neonatal MSUD screening assay was verified against other commonly occurring amino acids, metabolites and antibiotics. No Interference was observed from antibiotics, non-antibiotics and metabolites.

Method Comparison:

BornSafe™ Neonatal MSUD Screening Assay was compared with a CE certified commercial Neonatal MSUD Assay kit using Normal routine new born screening dried blood spots samples. Total 300 no of samples were tested in comparison in both assays. The range of MSUD concentration was 1.04 mg/dl to 3.65 mg/dl. Excellent Correlation was achieved between two NBS MSUD Assays.

16. LIMITATIONS

- Born Safe™** Neonatal MSUD Screening Assay is a screening method for the measurement of L-BCAAs in newborn blood spot specimens.
- Elevated results are not diagnostic per se of Maple Syrup Urine Disease but indicate the urgent need for further study of the newborn from which the 'presumptive positive' was received.
- Erroneous results may be generated due to poor blood sampling techniques or to technical/operational errors. Such errors may result in measured L-BCAA concentrations from dried blood spot specimens which are unrelated to the clinical status of the newborn, i.e. false positive or false negative results.

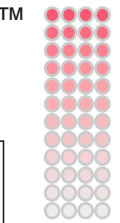
17. COMPLAINTS

Complaints can be accepted in written format (preferably on the manufacturer's complaint form). All details of the test kit, as well as the test results, can be included. A copy of the complaint form is available from Tulip Diagnostics Pvt Ltd. upon request.

18. REFERENCES

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Summary Protocol



REAGENT PREPARATION

| | | |
|----------------------------------|--|--|
| Reconstitution of Enzyme vial | | Each vial with 1 ml Distilled/Deionized water |
| Reconstitution of Co-Enzyme vial | | Each vial with 1 ml Distilled/Deionized water |
| Enzyme- Co-Enzyme solution | | 2 Parts Enzymes + 2 Parts Co-Enzyme + 1-Part Dilution Buffer |
| Colour Reagent Mixture | | 10 Parts Colour Reagent + 1-Part Colour Booster |

ASSAY PROCEDURE

| | | |
|---|--|--|
| 1. Punch out Calibrators, Controls and unknown in 'U' bottom microtiter plate | | 2 blood spots into each wells of round bottom microtiter plate |
| 2. Add Elution Buffer | | Add 100µl. Ensure that each disk is fully immersed in Elution Buffer |
| 3. Incubate | | 30 min at RT (20°-26°C) on an orbital plate shaker (900rpm) |
| 4. Transfer to corresponding well of flat bottom microtiter plate | | Add 40µl of the eluate from each well |
| 5. Add Enzyme-Co-Enzyme solution | | Add 100 µl. Gently mix, avoiding the formation of foam |
| 6. Incubate | | 30 min at RT (20°-26°C) |
| 7. Add Colour Reagent Mixture | | Add 80 µl. Gently mix, avoiding the formation of foam |
| 8. Incubate | | 10 min at RT, away from light |
| 9. Read/Measure | | Place the plate in a microplate reader and read at 550nm |

SYMBOL KEYS

| | | | |
|---------------------|------------------------------------|--|---------------------------|
| Store at 2°C to 8°C | Consult Instructions for use | Date of Manufacture | Batch Number / Lot Number |
| Manufacturer | In vitro Diagnostic Medical Device | This side up | Caution |
| Use by | Catalogue Number | Contains sufficient for $\leq n$ tests | |

Manufactured by:

Coral Clinical Systems
A Division of Tulip Diagnostics (P) Ltd.

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