

Neonatal Galactosemia (Total Galactose) Screening Assay



Enzymatic assay for the quantitative determination of Total Galactose levels in new born dried blood spots (FOR IN VITRO DIAGNOSTIC USE ONLY)

1. INTENDED USE

Born Safe™ Neonatal Total Galactose Screening Assay is an enzymatic assay for the quantitative determination of total galactose (galactose and galactose-1-phosphate) concentrations in neonates using blood spot samples dried on Whatman S&S 903 filter collection paper. This kit is particularly suitable for use in a neonatal screening program to measure concentrations as an aid in identifying galactosemia in newborns. Elevated results are not diagnostic per se of galactosaemia, but indicate the urgent need for further study of the newborn from which the 'presumptive positive' specimen was received. This kit is not intended for use in monitoring the circulating concentrations of total galactose in galactosaemic patients nor for confirmatory testing.

2. SUMMARY AND EXPLANATION OF THE ASSAY

Galactosemia is the term for a range of heterogenous autosomal recessive inherited metabolic defects revealed by screening newborn total galactose concentration.¹ The disease is characterised by elevated circulating analyte concentrations, galactose and galactose-1-phosphate,¹ due to the absence of one (or more) enzymes in the metabolic pathway concerned with the catabolism of the analytes.

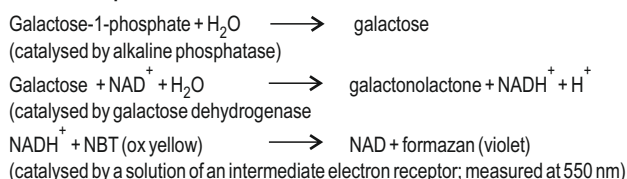
α -D-galactose is metabolized via a series of sequential reactions called Leloir pathway. The three enzymes that catalyse these reactions are galactokinase (GALK), galactose-1-phosphate uridylyltransferase (GALT),² and UDP-galactose 4'-epimerase (GALE). Deficiency of any one of these enzymes in humans results in a form of the inherited metabolic disorder, galactosemia. The most common and clinically severe form of galactosemia is classic galactosemia which results from insufficient galactose-1-phosphate uridylyltransferase (GALT), one of the three galactose metabolism enzymes. The measurement of galactose and galactose-1-phosphate concentration in dried blood spots is done to screen for deficiency in any of the three enzymes of galactose metabolism.

As a result, galactose accumulates in the blood, causing a variety of symptoms. If not diagnosed and treated within the newborn period, it can lead to diarrhoea, dehydration, jaundice, metabolic cirrhosis of the liver, kidney damage, hypoglycemia, cataracts, developmental retardation and death within a few weeks.³ Sepsis due to Escherichia coli seems to be particularly frequent among galactosemic neonates and is usually the cause of death.⁴ Early treatment with a dietary galactose/lactose restriction should be initiated promptly. Treatment of the disease consists of withdrawal of all foods containing lactose and galactose from the diet. Its rate of incidence is approximately 1 in 50 000 newborns worldwide.⁵

3. PRINCIPLE OF THE ASSAY

The Total Galactose (galactose and galactose-1-phosphate) from cellulose paper (dried blood spot samples) is extracted with trichloroacetic acid (Elution buffer). After extraction, the eluted sample is combined with the enzyme-coenzyme reagent containing alkaline phosphatase (AP), galactose dehydrogenase (GaldeH) and NAD. Galactose-1-phosphate is converted to galactose by AP. GaldeH oxidizes the galactose to galactonolactone reducing NAD to NADH. 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 total galactose present in the sample.

Reaction Sequence



4. PRESENTATION

REF	
1122040096	96 Assays

5. KIT COMPONENTS:

Reagents: (96T Pack size)

- Calibrators and Controls blood spots:** 1+1 set of blood spot cards of human whole blood spotted on Whatman S&S 903 paper containing 5 calibrators and 2 controls. 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.
- Enzyme 1 Reagent:** 1 x 0.1 ml of Alkaline phosphatase with buffer and a stabilizer.
- Enzyme 2 Reagent:** 1 x 0.5 ml of galactose dehydrogenase.
- Coenzyme Reagent:** 4 x 1.5 ml of Lyophilized NAD. Reconstitute with 1.5 ml of distilled water each. After reconstitution, the reagent can be stored at 2-8°C for one month.
- Dilution Buffer:** 1 x 5.0 ml of buffer. Preservative NaN_3 (< 0.1%). If a precipitate is

observed, put the solution few minutes at 37°C.

- Colour Reagent:** 1 x 8.0 ml of tetrazolium salt. Preservative: NaN_3 (< 0.1%).
- Colour Booster:** 1 x 1.0 ml of a solution of an intermediate electron receptor in buffer. Preservative NaN_3 (< 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 1 Reagent	1 x 0.1 ml	Ready to use
Enzyme 2 Reagent	1 x 0.5 ml	Ready to use
Coenzyme Reagent	4 x 1.5 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 5.0 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 the kit and reagents at 2-8°C. 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.

- Avoid splashing and aerosol formation; in case of spilling, wash carefully with a 3% sodium hypochlorite solution and dispose of this cleaning liquid as potentially infectious waste.
- 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.
- 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 skillful collecting, handling and transport of samples. The collection technique is described in detail in CLSI document LA4-A5,⁶ 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) 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.
- 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: Hemoglobin concentrations ≤ 250 g/L do not interfere with the test. Abnormally high glutathione concentration (≥ 60 mg/dL) can cause a clinically significant decrease in the apparent concentration of galactose. Lipemic samples also interfere with the test, causing an increase in the apparent concentration of galactose. With high lipid concentrations, there is a minor risk of false presumptive positive results. Blood specimens that have been obtained from infants who have not ingested sufficient breast milk or formula containing lactose may give low values.

*Special attention must be paid to the storage and transportation conditions of the DBS samples. Storage of samples in an environment with elevated temperatures and humidity increases the risk of false negative screening results.

10. REAGENTS PREPARATION

ENZYME-COENZYME SOLUTION

A. Reconstitution: First reconstitute, one Coenzyme vial with 1.5 ml of distilled or de-ionized water. Mix gently to aid reconstitution. After reconstitution, the reagent can be stored at 2-8° C for one month.

B. Preparation:

- Mix 1 part of Enzyme 1 with 5 parts of Enzyme 2, 50 parts of Coenzyme and 50 parts of dilution buffer (see table).

The following table gives the volumes required for each of the four components to run specific number of tests.

No. of Tests	Enzyme 1 (µl)	Enzyme 2 (µl)	Coenzyme (µl)	Dilution Buffer (µl)
10	10	50	500	500
20	20	100	1000	1000
40	40	200	2000	2000
80	80	400	4000	4000
100	100	500	5000	5000

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. 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.

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 mixing 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.

11. ASSAY PROCEDURE

A. ELUTION STEP:

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

B. SAMPLE TRANSFER AND ASSAY:

- 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
- Pipette **100 µl** of the Enzyme-Coenzyme solution prepared in section 10 to each well. Mix well, avoiding the formation of foam
- Incubate **30 minutes** at room temperature (20-26°C).
- Add **80 µl** of Colour Reagent Mixture prepared in section 10 to each well. Mix well to avoid the formation of foam.
- After **10 minutes** of incubation at room temperature keeping the plate away from light, measure the absorbance at 550-570 nm (optimal: 550 nm), endpoint mode, single measurement.

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.

C. CERTIFICATION/TRACEABILITY TO REFERENCE MATERIAL

Currently there are no Reference Materials available for Total Galactose.

12. CALCULATION OF RESULTS

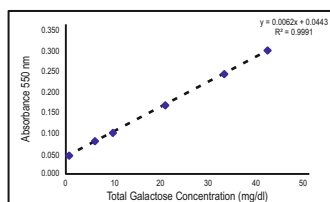
Draw a calibration curve, by plotting the calibrators concentration (x-axis) against the absorbance OD obtained for each calibrator (y-axis). The obtained OD of the calibrators are plotted against their concentration. The calibration curve is calculated by a linear regression function. Using computer programs, the curve is best described by a 2-points linear regression fit with linear axes. Corresponding galactose concentrations in mg/dL are obtained by interpolating the absorbances of each sample on the calibration curve.

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	5.7	0.082
C2	9.5	0.103
C3	20.4	0.167
C4	32.5	0.245
C5	41.2	0.305

Figure 1



13. QUALITY CONTROL

Control samples should always be used to assure the day-to-day validity of the test according to manufacturer. The controls should be run in the same way as the samples. These controls provide valuable information regarding the validity of the test according to manufacturer. The assay is acceptable if the concentrations for each Blood Spot Control are within the ranges quoted on the card label. The assay is unacceptable if the measured values for either of the Blood Spot Controls fall out of specification. If the assay is unacceptable, patient specimen results should not be reported. Each laboratory should establish its own mean and acceptable range. It is recommended that the laboratories establish their own controls at different levels in addition to the controls included in the kit. The stability of and storage conditions for the additional controls and the criteria for assay acceptance/rejection should be determined by each laboratory. The participation in National External Quality Assurance Schemes is strongly recommended. As part of good Quality Assurance practice, it is recommended that repeat testing be considered periodically where patient results register less than the detection limit of the assay in case these represent mis-sampling or procedural errors.

14. EXPECTED VALUES AND INTERPRETATION CRITERIA

The determination of presumptive positives for galactosemia is based on the use of a cut-off value which distinguishes between presumptive negative and presumptive positive results. Presumptive positives for galactosemia should be confirmed by a confirmatory test procedure i.e. galactose 1-phosphate uridylyl transferase (GALT).

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 should 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.

A review of various published NBS programs study outcomes and referring to the CDC-Newborn Screening Quality Assurance Program (NSQAP) summarized PT reported cut off values, following guidelines are suggested.

Suggested Cut off Values

Interpretation	Concentration (mg/dl)
Presumptive Negative	<10 mg/dl
Borderline	10- 20 mg/dl
Presumptive Positive	>20 mg/dl

15. ANALYTICAL PERFORMANCE CHARACTERISTICS

Precision: The intra assay and inter assay precision were determined as per NCCLS Evaluation Protocol (EP5-T2). 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 three different runs.

Specimen	N	Mean (mg/dl)	SD	Intra Assay Variation (CV%)
Control	10	20.3	0.42	2.06
Sample1	20	4	0.48	12
Specimen	N	Mean (mg/dl)	SD	Inter Assay Variation (CV%)
Sample 2	15	5.5	0.54	9.81
Sample 3	15	7.4	0.45	6.08

Precision study using CDC-PT material:

Sample	N	Target Value (mg/dl)	Mean (mg/dl)	SD	Inter-Assay Variation (CV%)
Blinded Sample 1	8	2.9	3.29	0.45	13.67
Blinded Sample 2	8	26	21.31	1.14	5.34

Analytical Sensitivity: Analytical Sensitivity was calculated by mean concentration of low levels samples (n=100) which can be statistically distinguished from '0' mg/dl. The Analytical Sensitivity of **BornSafe™** Neonatal TGAL Screening Assay is 1.3 mg/dl.

Interference:

The assay specificity of **BornSafe™** Neonatal Total Galactose screening assay was verified by deriving the concentration of Total Galactose along with other cross-reactive components. No Interference was observed from antibiotics, non-antibiotics and metabolites.

Method Comparison:

BornSafe™ Neonatal TGAL Screening Assay was compared with a CE certified commercial Neonatal TGAL Assay kit using few positive and Normal routine new born screening dried blood spots samples. Total 305 nos of samples were tested in comparison in both assays. Seven positive samples were screened in agreement with both assay systems. The range of TGAL concentration was 0.669 mg/dl to 34 mg/dl. Excellent Co-relation was achieved between two NBS Assays.

Recovery:

CDC-QC samples were used to establish the recovery of Galactose at several concentrations. These samples were run triplicates in five different runs (n=15)

Sample	N	Mean TGAL Target Value (mg/dl)	Mean TGAL Recovered (mg/dl)	Mean Recovery %	Range Recovery%
Low A	15	5.6	5.81	103	89%-113%
Medium B	15	10.7	10.84	101	99%-111%
High C	15	26.3	25.40	97	90%-109%

16. LIMITATIONS

Born Safe™ Neonatal Total Galactose Screening Assay is a screening method for the measurement of total D galactose in newborn dried blood spot specimens. Elevated results are not diagnostic per se of galactosaemia but indicate the urgent need for further study of the newborn from which the 'presumptive positive' was received. As with any other in vitro screening test, the data obtained using the Neonatal Total Galactose kit should be used as an aid to other medically established procedures and results interpreted in conjunction with other clinical data available to the clinician. A confirmatory laboratory procedure should be used to confirm elevated galactose and galactose-1-phosphate levels. An increased incidence of false negative results may be expected when the specimen is collected less than 48 hours after birth or the newborn did not receive an adequate lactose-containing diet or ingested sufficient breast milk prior to sampling. An increased incidence of false positive results may occur in the analysis of specimens from heterozygotes due to reduced galactose clearance capacity. All such potential false negative or false positive results are not device related. Erroneous results may be generated due to poor blood sampling techniques, or to technical/operational errors that may result in measured total galactose concentrations from dried blood spot specimens which are unrelated to the clinical status of the newborn, i.e. false positive or false negative results.

Conditions which are known to cause anomalous analytical assay results are: sample spot not uniformly saturated with blood:

- sample spots punched too close to the edge of the blood spot.
- poorly collected and improperly dried specimens.
- non-eluting blood spot due to deterioration of sample caused by exposure to heat and humidity.
- contamination of blood spot filter paper with faecal material.

Abnormally high glutathione concentration (≥ 60 mg/dL) can cause a clinically significant decrease in the apparent concentration of galactose. Lipemic samples also interfere with the test, causing an increase in the apparent concentration of galactose. With high lipid concentrations, there is a minor risk of false presumptive positive results. In all scenarios outlined above, if a false negative or false positive result is suspected the necessary follow-up procedures should be instigated immediately.

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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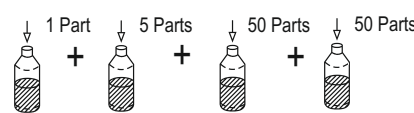
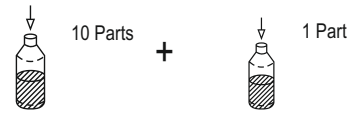
Summary Protocol

Born Safe
T.GAL


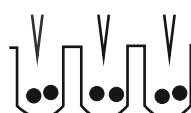


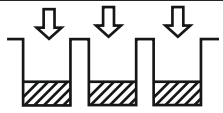
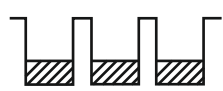
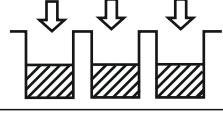


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










REAGENT PREPARATION

Reconstitute Co-Enzyme Vial		Each vial with 1.5 ml distilled /deionized water
Enzyme- Co-Enzyme solution		1-Part Enzyme 1 + 5 Parts Enzyme 2 + 50 Parts Co-Enzyme + 50 Parts 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 <n> tests	

Manufactured by:

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

M-46, Bldg. No. D, Phase IIIB, Verna Industrial Estate, Verna, Goa - 403 722, INDIA.
Regd. Office: Gitanjali, Tulip Block, Dr. Antonio Do Rego Bagh, Alto Santacruz, Bambolim Complex P.O., Goa - 403 202, INDIA.