INTENDED USE
The kit has been designed for the semi-quantitative in vitro determination of biotinidase activity in the screening of neonatal blood spots.

PRINCIPLE OF THE ASSAY
Biotinidase is found in the blood sample itself. Filter paper disks from newborn dried blood spot samples, calibrators and controls are punched into the wells of a microplate. When biotin substrate reagent containing Biotinyl-4-aminobenzoic acid is added to a well containing a punched dried blood spot, the reagent extracts and reconstitutes the proteins and enzymes in the spot. The biotinidase enzyme in the sample cleaves the substrate to biotin and 4-aminobenzoic acid. The addition of the TCA reagent stops the reaction and precipitates the proteins to cover the bottom of the well and the extracted spot. End of the reaction PABA is formed. Following the addition of color reagent the specific color is read by photometer (570 nm).

KIT CONTENTS
All reagents of the kit (except for the controls and calibrators) are stable until the expiry date indicated on the kit label, if stored at 2-8°C.

1- Biotinidase Substrate Reagent:
Buffered solution of Biotinyl-4-aminobenzoic acid. Ready to use 60 ml NBC500, 120 ml NBC1000

2- TCA Reagent:
Trichloroacetic acid solution. Ready to use. 60 ml NBC500, 120 ml NBC1000

3- Color Reagent 1:
Sodium Nitrite Solution. Ready to use. 20 ml NBC500, 40 ml NBC1000

4- Color Reagent 2:
Ammonium Sulphamate Solution. Ready to use. 20 ml NBC500, 40 ml NBC1000

5- Color Reagent 3:
Naphthyl ethylene diamine solution. Ready to use. 20 ml NBC500, 40 ml NBC1000

6- Reaction plates: 5 pcs - 10 pcs
Non coated polystrene microplates

7- Calibrators and controls:
Paper with Calibrators (6 levels) and Controls (2 levels) in a foil package with a desiccant. They were prepared by pooling blood from the normal population.
The values of the calibrator’s are indicated as % Activity and the Unit on the calibrator sheet in the kit.
The calibrators are stable 6 months if they stored at -20 °C

! Keep the calibrators and controls - 20 °C

8- Adhesive slips: 10 pcs - 20 pcs

OTHER MATERIALS REQUIRED, BUT NOT PROVIDED
- Elution plate. U bottom microplate for disk elution.
- Disk puncher with a diameter of 3 mm.
- Precision micropipettes 50 -200 µl, 200-1000 µl. 8-channel micropipette 50-300 µl.
- Microplate centrifuge.
- Incubator
- Microplate Photometer

PRECAUTIONS AND WARNINGS
- This reagent kit is for in vitro diagnosis only.
- This reagent kit is for professional use only.
- Do not use reagent past its expiration date.
- Do not interchange reagents between different lots.
- TCA reagent and Color Reagent 3 are corrosive. Protective gloves should be worn while using this reagent. Avoid skin contact.
- All kit components and specimens should be regarded as potential hazards to health. It should be used and discarded according to your own laboratory’s safety procedures. Such safety procedures probably will include the wearing of protective gloves and avoiding the generation of aerosols.

SPECIMEN COLLECTION AND HANDLING
The specimen collection technique is described in detail in NCCLS document LA4-A3. The samples of blood are collected and dried on the filter paper reserved for neonatal screening test (Schleicher & Schuell 903). The blood sample is collected between 3-5 days after birth from newborn’s heel. The heel is cleaned with 70% alcohol and punctured with a sterile blood lancet. The blood drop obtained is soaked onto the filter paper in the center of the circle printed. Both sides of the paper have to be penetrated and saturated by this one drop.

After collection of the samples, the filter papers are dried horizontally for 2-3 hours at room temperature. The dry samples should be stored at -20 °C. The dry specimens can be stored at -20°C for at least 2 years under desiccated conditions.

Test procedure:
1- Place 6 mm (or 2 x 3mm) punched spots into the appropriate well on the Elution plate (U bottom plate).
2- Reserve 6 wells for calibrators and 2 wells for controls
3- Add 100 µl Biotinidase Substrate Reagent to all wells
4- Cover the plate with adhesive slip and incubate for 4 -6 hours at 37°C
5- End of the incubation add 100 µl of cold TCA Reagent to all wells and cover the plate. (Leave TCA reagent at 2-8°C before pipetting)
6- Cover the plate with adhesive slip then place on the microplate centrifuge and rotate for 5 minutes at 4000 RPM.
7- Transfer 100 µl of supernatant from Elution plate into the correct wells of the Reaction plate. Please note that do not take residues.
8- Pipette 30 µL of Color Reagent 1 into all wells of Reaction Plate.
9- Mix and incubate for 5 minutes at room temperature (20-25°C).
10- Pipette 30 µL of Color Reagent 2 into all wells of Reaction Plate.
11- Mix and incubate for 5 minutes at room temperature (20-25°C).
12- Pipette 30 µL of Color Reagent 3 into all wells of Reaction Plate.
13- Mix and incubate for 10 minutes at room temperature (20-25°C).
14- Read the absorbance within 60 min using microplate photometer Filter; 570 nm.

LIMITATIONS
The test should not be used to detect biotinidase activity in prematurely born infants or those being treated with sulfonamides or like compounds. Abnormally high blood albumin concentrations can produce biotinidase activity higher than the actual level.
RESULTS
Calculation of the Results
Results are obtained from the standard curve by interpolation. The curve serve for the determination in samples measured at the same time as the calibrators. Calculation of results can be carried out manually if there is no automatic data reduction.

Figure 1: Example of calibration curve

If automatic data processing can be used, linear or cubic spline curve fitting with lin-lin axis scaling is recommended. Determine the absorbance for each well. Plot the calibrator curve using linear graph paper with concentration of calibrators on the x-axis and absorbance on the y-axis.

EXPECTED VALUES
The biotinidase activity measured by the ODAB™ Biotinidase Assay Kit was assessed in a study by testing 1750 neonatal dried blood spot specimens. It is generally accepted that normal and abnormal biotinidase activity range are as follow:

- **Deficient** < 10%  Mean Normal Activity
- **Partially Deficient** = 10-30%  Mean Normal Activity
- **Normal** > 30%  Mean Normal Activity

However, it is strongly recommended that each laboratory determines its own reference ranges and cut-offs based on specimens from the laboratory routine population. A procedure should also be established for the close follow-up of biotinidase deficient or partially deficient cases. Samples considered abnormal should have confirmatory testing performed without delay. This kit method should only be used as an initial screening tool.

PERFORMANCE CHARACTERISTICS
Intra-assay Precision
Controls (n = 22) in the normal range typically showed %CV of less than 10%.

Inter-assay Precision
Controls (n = 10 runs) in the normal range typically showed %CV of less than 10%.

Sensitivity
The sensitivity of the assay is typically less than 5 U. The sensitivity is defined as that concentration of analyte which corresponds to the dose response variable (OD) that is two standard deviations from the extrapolated zero of 10 replicate determinations of the zero calibrator run in a single assay.

REFERENCES:
4. Determination of Biotinidase Activity with Biotinyl-6-aminoquinoline as Substrate By Kou HAYAKAWA, KAZUYUKI YOSHIKAWA, JUN OIZUMI, METHODS IN ENZYMOLOGY, VOL. 279
5. Technical calibrators and guidelines for the diagnosis of biotinidase deficiency ACMG CALIBRATORS AND GUIDELINES

Product No:
- 480 tests REF: NBC500
- 960 tests REF: NBC1000

MANUFACTURER:
ODAK Neonatal Biotinidase Assay
Assay Rev 02 : 25.08.2013

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