ODAK Neonatal Fluorometric Biotinidase Assay

Invitro fluorometric determination of biotinidase activity in dry blood spots

Product No: NFB1000

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 biotin 6-aminoquinoline (6-AQ) 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 fluorescent 6-AQ. The addition of the ethanol-acetone stops the reaction and precipitates the proteins to cover the bottom of the well and the extracted spot. The fluorescent product (6-AQ) formed during the reaction is measured with a fluorometer.

Biotinidase
biotin 6-AQ → biotin + 6-AQ

Excitation wavelength is 360 nm and the emission wavelength is 480 nm. The biotinidase activity of the sample is determined by comparing the fluorescence intensity of the sample to a calibration curve.

KIT CONTENTS
All reagents of the kit are stable until the expiry date indicated on the kit label, if stored at 2-8°C.

1-Biotinidase buffer : 65 ml
Ready to use. Contains Bronidox 0.2% as preservative.

2- Reaction plates: 10 pcs
Non coated white microplates.

3- Calibrators:
Paper with 4 sets of 6 calibrators, 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

6AQ equivalent of 1U = nmol/min/100 ml.

OTHER MATERIALS REQUIRED, BUT NOT PROVIDED
- Ethanol-acetone
- 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 fluorometer

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.
- Ethanol and acetone is highly flammable. Keep container tightly closed. Keep away from sources of ignition.
- 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.

STORAGE CONDITIONS
- All components must be stored at 2 to 8°C (except for standards)
- All the components of the kit are stable until the expiration date on the label when stored tightly closed at 2-8°C, protected from light and contaminations prevented during their use.
- Do not use reagents over the expiration date.
- The calibrators should be stored at -20°C.

ASSAY PROCEDURE
- Mix ethanol and acetone as 1/1 volume and store at -20°C.

Test procedure:
1- Place 3 mm punched spots into the appropriate well on the Elution plate.
2- Reserve 6 wells for standards.
3- Add 60 μl test mixture to each well, cover the plate and incubate for 4 hours at 37°C.
4- Add 180 μl of cold ethanol-acetone mixture all wells and cover the plate.
5- Place the plate on the microplate centrifuge and rotate for 5 minutes at 4000 RPM.
6- Transfer 100 μl of supernatant from Elution plate into the correct wells of the White plate.
7- Read the fluorescence within 10 min using microplate fluorometer

Filters; excitation 360 nm/emission 480 nm.
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 F.I. for each well. Plot the calibrator curve using linear graph paper with concentration of calibrators on the x-axis and F.I. on the y-axis.

EXPECTED VALUES
The biotinidase activity measured by the ODAK Biotinidase Assay Kit was assessed in a study by testing 1750 neonatal dried blood spot specimens.

It is generally accepted that abnormal biotinidase samples are as follows:

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.

Measurement range:
From 5 IU to 250. The calibration curve is linear between lowest and highest calibrators.

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 (F.I.) that is two standard deviations from the extrapolated zero of 10 replicate determinations of the zero calibrator run in a single assay.

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.

REFERENCES:
5. Determination of Biotinidase Activity with Biotinyl-6-aminoquinoline as Substrate By Kou HAYAKAWA, KAZUYUKI YOSHIKAWA, JUN OIZUMI, METHODS IN ENZYMIOLOGY, VOL. 279
6. Technical standards and guidelines for the diagnosis of biotinidase deficiency ACMG STANDARDS AND GUIDELINES
7. A quantitative fluorometric micromethod used for the neonatal screening of biotinidase deficiency in Finland Screening, 1 (1992) 185-194 Lahja Pitktnen” and Tamara Tuuminen

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