GALACTOSE
Screening assay

Enzyme colorimetric assay for the quantitative determination of Total Galactose levels in newborns

Using DRIED BLOOD SPOTS

CE

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Indications

- Quantitative measurement of total galactose (galactose and galactose-1-phosphate) concentrations in dried blood spot collected onto Schleicher & Schuell (S&S®) 903™ specimen collection paper.
- Particularly suitable for use in a newborn screening program to measure total galactose concentrations in newborn infants as an aid for the early detection of Galactosemia.

Features

- Convenient transport and good stability of the samples
- Accurate, sensitive, rapid and specific assay
- Reading at 550 nm

Kit contents

Reagents	Quantity 500 tests	Quantity 2000 tests
Enzyme I	1 x 110 µl	1 x 440 µl
Enzyme II	1 x 1.8 ml	1 x 7.5 ml
Coenzyme	4 x 9.8 ml	4 x 39 ml
Dilution buffer	1 x 10.5 ml	1 x 42 ml
Colour Reagent	1 x 43 ml	1 x 175 ml
Colour Reagent Booster	1 x 4.3 ml	1 x 17.5 ml

ORDERING INFORMATION

*Code : **E-HQ-500***
Package Size : 500 tests/kit

*Code : **E-HQ-2000***
Package Size : 2000 tests/kit

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Simple assay procedure

1. Take a **clean** 96-well (preferably **U** bottom) microplate **(elution microplate).**
2. Add one disk cut from a dried blood spot **(4.7 mm or 2 X 3.2 mm diameter)** per well. Remember to add **controls, standards and one blank well.**
3. Warm up all reagents (except the color reagent) to room temperature.
4. Add 100 µl of Elution Buffer (TCA 3%) in each well, **mix well** the contents of each well and place the plate on a plate shaker.
5. Wait **30 minutes** at room temperature (20-26 °C).
6. While waiting reconstitute one Coenzyme Vial with **39 ml distilled water.** The dilution buffer, Enzyme 1 and Enzyme 2 reagents are ready to use. Mix the four reagents according to the table below in correlation to the number of tests to be run. Shake well the enzyme vials to resuspend all matter. We strongly recommend that the dilution buffer be added to the mixture just before use. You need 100 µl of this Enzyme 1-Enzyme 2 – Coenzyme – Dilution buffer mixture for each sample. Please note that you should only mix the quantity you need for the day's run. The Enzyme 1 – Enzyme 2 – Coenzyme mixture should be discarded if not used within 8 hours.

# tests	Enzyme I (µl)	Enzyme II (µl)	Coenzyme (ml)	Dilution buffer	Total Volume (ml)
50	10	182	3.845	1	5
100	20	365	7.68	2	10
150	30	546	11.52	3	15
200	40	729	15.36	4	20
250	50	911	19.2	5	25
300	60	1092	23	6	30
350	70	1274	26.93	7	35
400	80	1461	30.73	8	40
450	90	1643	34.56	9	45
500	100	1825	38.4	10	50
1000	200	3650	76.8	20	100
2000	400	7300	153.6	40	200

7. Transfer **40 µl** of the TCA eluant in a new microplate at the corresponding wells. Add 100 µl of the mixture prepared in step 6 per well. Mix well, avoiding the formation of foam. Wait for **30 minutes** at room temperature (20-26 °C).
8. Take the Color Reagent and the Color Reagent Booster out of the refrigerator, mix one part of Color Reagent Booster with 10 parts Color Reagent just before using it. Do not pre-warm the mixture. Return the original bottles back to the refrigerator the soonest possible. Avoid exposure to light. Prepare only the quantity you will need for the day.
9. Add **80 µl** of Color Reagent mixture per well. **Mix well** avoiding the formation of foam. Always bring it back to the refrigerator when the assay is over.
10. Wait for **10 minutes** and measure the microplate at **550 nm,** endpoint mode, single measurement. **There is no need to wait longer than 15 minutes**. If this stage is prolonged, (i.e. more than 20 minutes) a high background may be observed.
11. Calculate the slope and the sample values.

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