



# MSUD

## Screening assay

**Enzyme colorimetric assay  
for the quantitative determination  
of Maple Syrup Urine Disease  
in newborns**



ZenTech

### ORDERING INFORMATION

Code : **E-HP-500**

Package Size : 500 tests/kit

Code : **E-HP-2000**

Package Size : 2000 tests/kit



### Indications

- Quantitative detection of Maple Syrup Urine Disease in newborn using dried blood spots.

### Features

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

### Kit contents

Reagents	500 tests	Quantity	2000 tests
Enzyme	4 x 5 ml		4 x 20 ml
Coenzyme	4 x 5 ml		4 x 20 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



Zentech s.a.

Liège Science Park

Avenue du Pré-Aily,10

B-4031 Angleur • Belgium

Tel : +32 (0)4 361 42 32

Fax : +32 (0)4 367 00 63

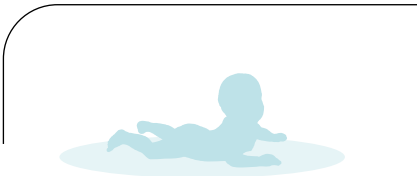
info@zentech.be

http://www.zentech.be



ZenTech

**NEONATAL SCREENING**



# MSUD

## Screening assay



### 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 Enzyme and one Coenzyme vial with distilled water. Each vial should be reconstituted with 20 ml of distilled water. Stable for one week refrigerated. Mix 2 parts of Enzyme solution with 2 parts of Coenzyme solution and 1 part of Dilution buffer. You need 100 µl of this Enzyme-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-Coenzyme mixture should be discarded if not used within 5 hours. The following table gives the volumes required from each of the three components to run specific number of tests (volumes in ml). We highly recommend the addition of the Dilution buffer just before using the mixture.

# tests	Enzyme (ml)	Coenzyme (ml)	Dilution buffer (ml)	Total Volume (ml)
50	2	2	1	5
100	4	4	2	10
150	6	6	3	15
200	8	8	4	20
300	12	12	6	30
400	16	16	8	40
500	20	20	10	50

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 and 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.
10. Wait for **10 minutes** and measure the microplate at **550 nm**, endpoint mode, single measurement. **There is no need to wait longer than 20 minutes**.
11. Calculate the slope and the sample values.

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info@zentech.be  
<http://www.zentech.be>