

Enzymatic measurement of **CITRATE** content

<Reagents>

1) Assay buffer ; 1M Tris-HCl (pH 7.8@25°C) with MgSO₄ and ZnSO₄

	1 M Tris-HCl	100 mM MgSO ₄ · 7H ₂ O	100 mM ZnSO ₄ · 7H ₂ O
preparation	12.1 g / 100 mL	1.23 g / 50 mL	1.44 g / 50 mL
for 40 spls	5 mL	15 µL	15 µL
for 60 spls	7.5 mL	22.5 µL	22.5 µL
for 80 spls	10 mL	30 µL	30 µL
for 100 spls	12.5 mL	37.5 µL	37.5 µL

2) NADH solution ; NADH (Na₂ salt) in 90 mM NaHCO₃

	NADH	90 mM NaHCO ₃
preparation		0.76 g / 100 mL
for 40 spls	5.3 mg	0.67 mL
for 60 spls	8 mg	1 mL
for 80 spls	11.2 mg	1.4 mL
for 100 spls	6.65 mg × 2	0.835 mL × 2

3) MDH / LDH mixture

	MDH	LDH	Milli-Q
for 40 spls	26 µL	11 µL	51 µL
for 60 spls	40 µL	15 µL	69 µL
for 80 spls	53 µL	22 µL	101 µL
for 100 spls	66 µL	26 µL	120 µL

4) CL solution

	CL
preparation	120 U / 2.4 mL
for 40 spls	200 µL
for 60 spls	300 µL

for 80 spls	400 μ L
for 100 spls	500 μ L

5) Citrate solution for STD

	1 mM citrate
preparation	10 mg / 50 mL

citrate concentration (μ M)	0	5	10	20	50	100
citrate content (nmol)	0	5	10	20	50	100
1mM citrate (μ L)	0	5	10	20	50	100
Milli-Q (μ L)	1000	995	990	980	950	900

<Procedure>

1. Mix the following solutions in each cuvette (for UV)

0.7 mL Milli-Q 0.3 mL sample 0.12 mL assay buffer

2. Add 15 μ L NADH solution in 30 sec intervals and mix well

3. Incubate 40 min at room temperature

4. Add 2 μ L MDH / LDH mixture in 30 sec intervals and mix well

5. Incubate 40 min at room temperature

6. Read A_{340} in 30 sec intervals

7. Add 5 μ L CL solution in 30 sec intervals and mix well

8. Incubate 30 min at room temperature

9. Read A_{340} in 30 sec intervals