Supplementary Information (SI) for New Journal of Chemistry.

Supporting information

Fumarate production from pyruvate and low concentrations of CO_2 with the multi-enzymatic system in the presence of NADH and ATP

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1. Determination for L-malate concentration using ion chromatography (1)

The concentration of L-malate was determined using ion chromatography system (Metrohm, Eco IC; electrical conductivity detector) with an ion exclusion column (Metrosep Organic Acids 250/7.8 Metrohm; column size: 7.8 × 250 mm; composed of 9 μm polystyrene-divinylbenzene copolymer with sulfonic acid groups). The 1.0 mM perchloric acid and 50 mM lithium chloride in aqueous solution were used as an eluent and a regenerant, respectively. Flow rate of eluent solution was adjusted to be 0.5 mL min^{-1}. The retention time for L-malate was detected at 10.11-10.13 min. The electrical conductivity changes in the various L-malate concentrations (0 – 1.0 mM) during the ion chromatograph analysis were shown in Figure S1(a). Figure S1(b) shows the relationship between the L-malate concentration and the detection peak area using ion chromatograph.

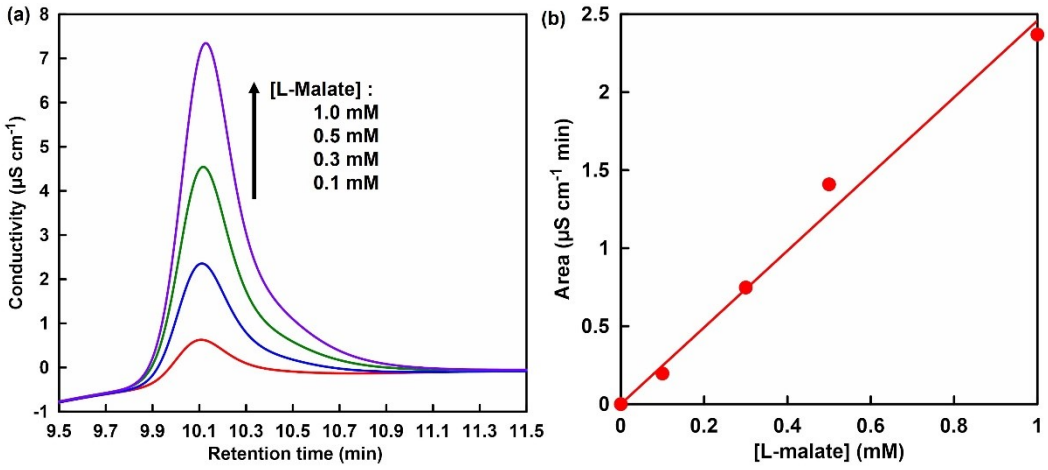

Figure S1. Chromatogram of sodium L-malate (0 – 1.0 mM) in 50 mM-HEPES buffer (pH 7.0) (a). Relationship between the L-malate concentration and the detection peak area (b).

As shown in the inset of Figure S1, the L-malate concentration and the detected peak area showed a good linear relationship (correlation coefficient: r^2=0.996) as following equation (S1).

$$\text{Peak area} = 2.5 \times [\text{L-malate}](\text{mM}) \quad \text{(S1)}$$

2. Determination for L-malate concentration using ion chromatography (2)

The concentration of L-malate was determined using ion chromatography system (Metrohm, Eco IC; electrical conductivity detector) with an ion exclusion column (Metrosep Organic Acids 250/7.8 Metrohm; column size: 7.8 × 250 mm; composed of 9 μm polystyrene-divinylbenzene copolymer with sulfonic acid groups). The 1.0 mM perchloric acid and 50 mM lithium chloride in aqueous solution were used as an eluent and a regenerant, respectively. Flow rate of eluent solution was adjusted to be 0.5 mL min^{-1}. The retention time for L-malate was detected at 9.93-10.3 min. The electrical conductivity changes in the various L-malate concentrations (0 – 5.0 mM) during the ion chromatograph analysis were shown in Figure S2(a). Figure S2(b) shows the relationship between the L-malate concentration and the detection peak area using ion chromatograph.

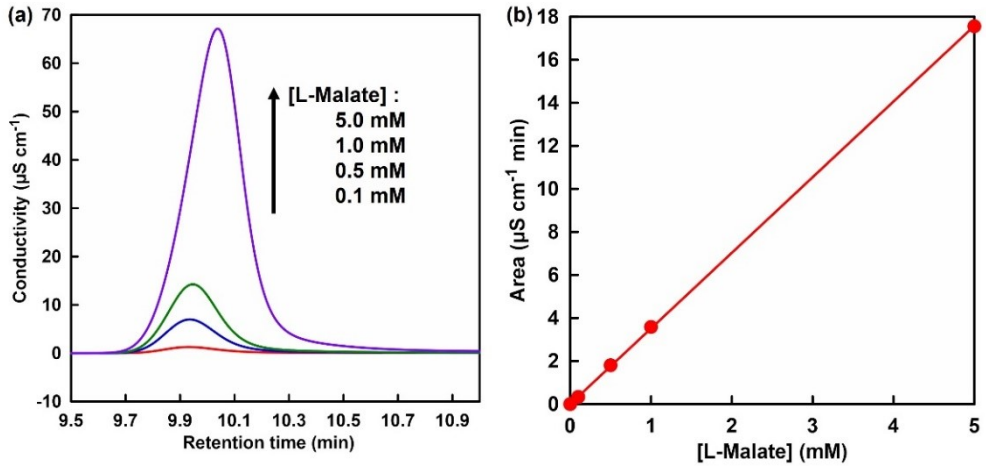

Figure S2. Chromatogram of sodium L-malate (0 – 5.0 mM) in 50 mM-HEPES buffer (pH 7.0) (a). Relationship between the L-malate concentration and the detection peak area (b).

As shown in the inset of Figure S2(b), the L-malate concentration and the detected peak area showed a good linear relationship (correlation coefficient: r^2=1.0) as following equation (S2).

$$\text{Peak area} = 3.5 \times [\text{L-malate}](\text{mM}) \quad \text{(S2)}$$

3. Determination for fumarate concentration using ion chromatography

The concentration of fumarate was determined using ion chromatography system (Metrohm, Eco IC; electrical conductivity detector) with an ion exclusion column (Metrosep Organic Acids 250/7.8 Metrohm; column size: 7.8 × 250 mm; composed of 9 μm polystyrene-divinylbenzene copolymer with sulfonic acid groups). The 1.0 mM perchloric acid and 50 mM lithium chloride in aqueous solution were used as an eluent and a regenerant, respectively. Flow rate of eluent solution was adjusted to be 0.5 mL min^{-1}. The retention time for fumarate was detected at 12.05-12.29 min. The electrical conductivity changes in the various fumarate concentrations (0 – 2.0 mM) during the ion chromatograph analysis were shown in Figure S3(a). Figure S3(b) shows the relationship between the fumarate concentration and the detection peak area using ion chromatograph.

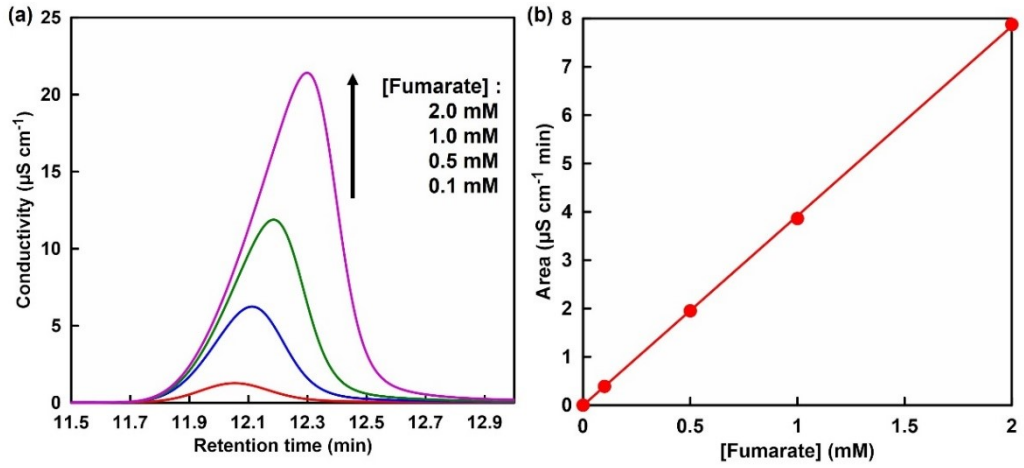

Figure S3. Chromatogram of sodium fumarate (0 – 2.0 mM) in 50 mM-HEPES buffer (pH 7.0) (a). Relationship between the fumarate concentration and the detection peak area (b).

As shown in the inset of Figure S3(b), the fumarate concentration and the detected peak area showed a good linear relationship (correlation coefficient: r^2=0.999) as following equation (S3).

$$\text{Peak area} = 3.9 \times [\text{fumarate}](\mu M) \quad \text{(S3)}$$

4. A chart of an ion chromatogram sampled from the reaction solution of sodium pyruvate, ATP, sodium bicarbonate, manganese chloride, NADH, PC, and MDH with varying concentrations of acetyl-CoA

Figure S4 shows an ion chromatography chart of a sample consisting of sodium pyruvate (2.0 mM), ATP (2.0 mM), sodium bicarbonate (50 mM), manganese chloride (5.0 mM), NADH (2.0 mM), PC (1.0 U), MDH (10 U) and acetyl-CoA in 5.0 mL of 500 mM HEPES buffer-NaOH (pH 7.2) after 30 min incubation. The concentration of acetyl-CoA was varied from 0.1 and 2.0 mM.

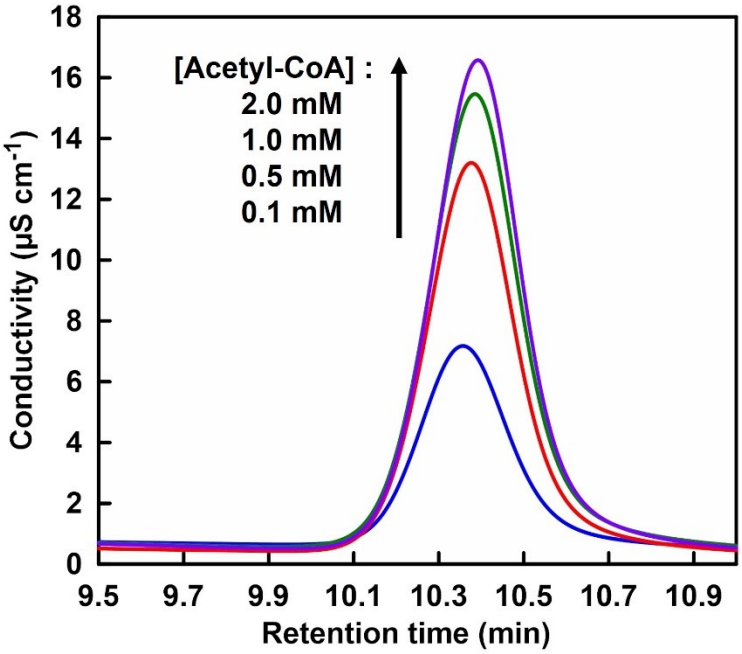

Figure S4. Chromatogram of a sample consisting of sodium pyruvate (2.0 mM), ATP (2.0 mM), sodium bicarbonate (50 mM), manganese chloride (5.0 mM), NADH (2.0 mM), PC (1.0 U), MDH (10 U) and acetyl-CoA (0.1 – 2.0 mM) in 5.0 mL of 500 mM HEPES buffer-NaOH (pH 7.2) after 30 min incubation.

5. A chart of an ion chromatogram sampled from the reaction solution of sodium pyruvate, ATP, manganese chloride, NADH, PC, MDH and acetyl-CoA sodium with varying concentrations of bicarbonate

Figure S5 shows an ion chromatography chart of a sample consisting of sodium pyruvate (2.0 mM), ATP (2.0 mM), sodium bicarbonate, manganese chloride (5.0 mM), NADH (2.0 mM), PC (1.0 U), MDH (10 U) and acetyl-CoA (1.0 mM) in 5.0 mL of 500 mM HEPES buffer-NaOH (pH 7.2) after 30 min incubation. The concentration of sodium bicarbonate was varied from 2.0 and 25 mM.

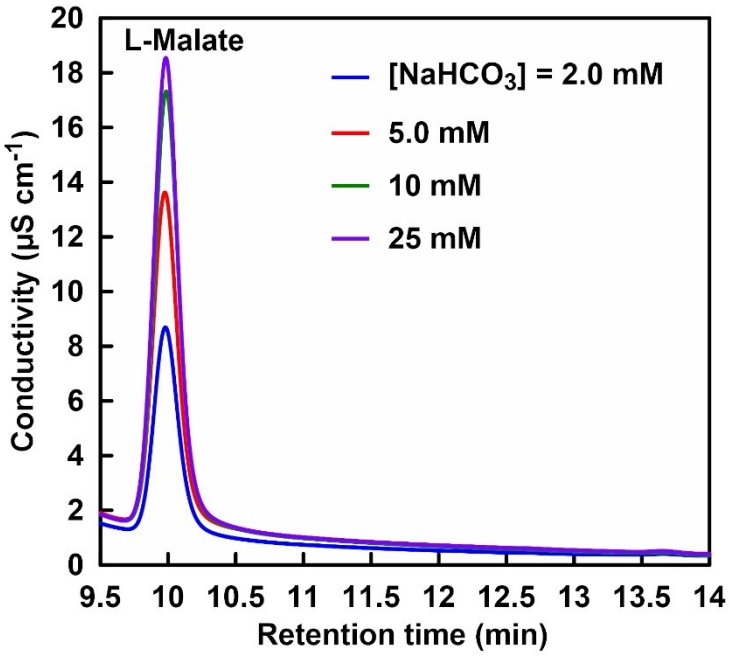

Figure S5. Chromatogram of a sample consisting of sodium pyruvate (2.0 mM), ATP (2.0 mM), sodium bicarbonate (2.0 – 25 mM), manganese chloride (5.0 mM), NADH (2.0 mM), PC (1.0 U), MDH (10 U) and acetyl-CoA (1.0 mM) in 5.0 mL of 500 mM HEPES buffer-NaOH (pH 7.2) after 30 min incubation.

6. L-Malate production from the pyruvate and direct captured CO_2 with PC and MDH in the presence of ATP and NADH

Figure S6 shows a chart of an ion chromatogram sampled from the reaction solution of sodium pyruvate (5.0 mM), manganese chloride (5.0 mM), ATP (5.0 mM), NADH (5.0 mM), acetyl-CoA (1.0 mM), PC (1.0 U) and MDH (10 U) in 5.0 mL of 500 mM HEPES buffer (pH 7.2) with a mixture gas of CO_2 and N_2 during the incubation. The composition of the gas phase including ballon was adjusted to 85 % N_2 and 15 % CO_2 (a) or 100% CO_2 gas (b). Figure S6(c) shows a chart of an ion chromatogram sampled from the reaction solution of sodium pyruvate (5.0 mM), magnesium chloride (5.0 mM), NADH (5.0 mM) and ME (0.7 U) in 5.0 mL of 500 mM HEPES buffer-NaOH (pH 7.2) with a mixture gas of 85 % N_2 and 15 % CO_2.

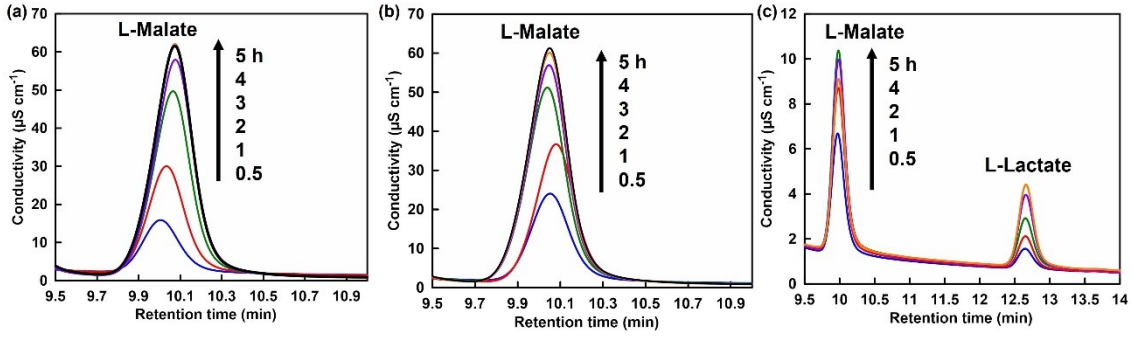

Figure S6. A chart of an ion chromatogram for L-malate concentration in the solution of sodium pyruvate (5.0 mM), manganese chloride (5.0 mM), ATP (5.0 mM), NADH (5.0 mM), acetyl-CoA (1.0 mM), PC (1.0 U) and MDH (10 U) in 5.0 mL of 500 mM HEPES buffer (pH 7.2) with a mixture gas of CO_2 and N_2 during incubation time. The composition of the gas phase including ballon was adjusted to 85 % N_2 and 15 % CO_2 (a) or 100% CO_2 gas (b). (c): A chart of an ion chromatogram sampled from the reaction solution of sodium pyruvate (5.0 mM), magnesium chloride (5.0 mM), NADH (5.0 mM) and ME (0.7 U) in 5.0 mL of 500 mM HEPES buffer-NaOH (pH 7.2) with a mixture gas of 85 % N_2 and 15 % CO_2 during incubation time.

7. Fumarate production from the pyruvate and direct captured CO_2 with MDH and FUM in the presence of ATP and NADH

Figure S7 shows a chart of an ion chromatogram sampled from the reaction solution of sodium pyruvate (5.0 mM), manganese chloride (5.0 mM), ATP (5.0 mM), NADH (5.0 mM), acetyl-CoA (1.0 mM), PC (1.0 U), MDH (10 U) and FUM (0.5 U) in 5.0 mL of 500 mM HEPES buffer (pH 7.2) with a mixture gas of CO_2 and N_2 during the incubation. The composition of the gas phase including ballon was adjusted to 85 % N_2 and 15 % CO_2 (a) or 100% CO_2 gas (b). Figure S7(c) shows a chart of an ion chromatogram sampled from the reaction solution of sodium pyruvate (5.0 mM), magnesium chloride (5.0 mM), NADH (5.0 mM), ME (0.7 U) and FUM (0.5 U) in 5.0 mL of 500 mM HEPES buffer-NaOH (pH 7.2) with a mixture gas of 85 % N_2 and 15 % CO_2.

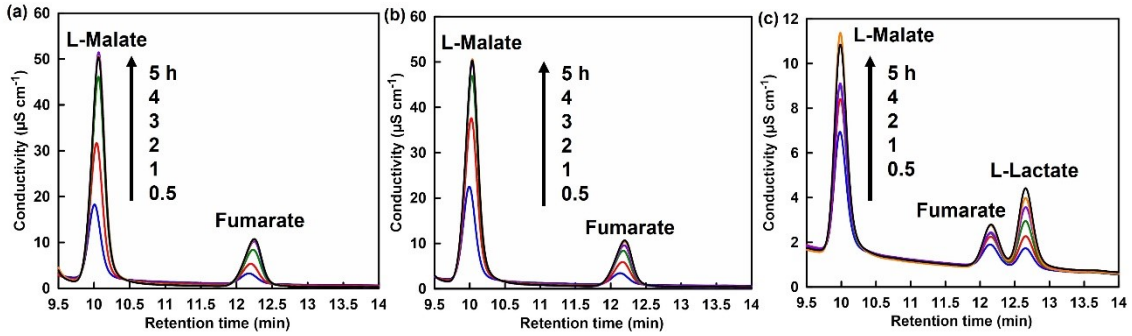

Figure S7. A chart of an ion chromatogram for L-malate concentration in the solution of sodium pyruvate (5.0 mM), manganese chloride (5.0 mM), ATP (5.0 mM), NADH (5.0 mM), acetyl-CoA (1.0 mM), PC (1.0 U), MDH (10 U) and FUM (0.5 U) in 5.0 mL of 500 mM HEPES buffer (pH 7.2) with a mixture gas of CO_2 and N_2 during incubation time. The composition of the gas phase including ballon was adjusted to 85 % N_2 and 15 % CO_2 (a) or 100% CO_2 gas (b). (c): A chart of an ion chromatogram sampled from the reaction solution of sodium pyruvate (5.0 mM), magnesium chloride (5.0 mM), NADH (5.0 mM), ME (0.7 U) and FUM (0.5 U) in 5.0 mL of 500 mM HEPES buffer-NaOH (pH 7.2) with a mixture gas of 85 % N_2 and 15 % CO_2 during incubation time.

Figure S8 shows a chart of an ion chromatogram sampled from the reaction solution of sodium pyruvate (5.0 mM), manganese chloride (5.0 mM), ATP (5.0 mM), NADH (5.0 mM), acetyl-CoA (1.0 mM), PC (1.0 U), MDH (10 U) and FUM (0.5 U) in 5.0 mL of 500 mM HEPES buffer (pH 7.2) with a mixture gas of CO_2 and N_2 during the incubation for 3 h. The composition of the gas phase including ballon was adjusted to 85 % N_2 and 15 %.

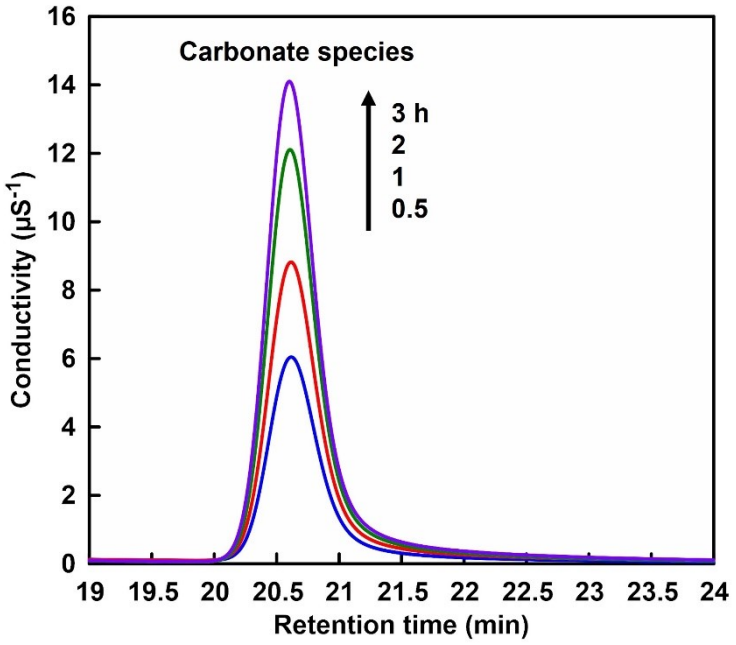

Figure S8. A chart of an ion chromatogram for L-malate concentration in the solution of sodium pyruvate (5.0 mM), manganese chloride (5.0 mM), ATP (5.0 mM), NADH (5.0 mM), acetyl-CoA (1.0 mM), PC (1.0 U), MDH (10 U) and FUM (0.5 U) in 5.0 mL of 500 mM HEPES buffer (pH 7.2) with a mixture gas of 85 % N_2 and 15 % CO_2 during incubation time.

8. Fumarate production from the pyruvate and direct captured CO_2 with MDH and FUM in the presence of ATP and NADH

Figure S9 shows a chart of an ion chromatogram sampled from the reaction solution of sodium pyruvate (5.0 mM), manganese chloride (5.0 mM), ATP (5.0 mM), NADH (5.0 mM), acetyl-CoA (1.0 mM), PC (1.0 U), MDH (10 U) and FUM (0.5 U) in 5.0 mL of 500 mM HEPES buffer (pH 7.2) with a mixture gas of CO_2 and N_2 after 2 h incubation. The composition of the gas phase including ballon was adjusted to 90 % N_2 and 10 % CO_2, 85 % N_2 and 15 % CO_2 and 100% CO_2 gas.

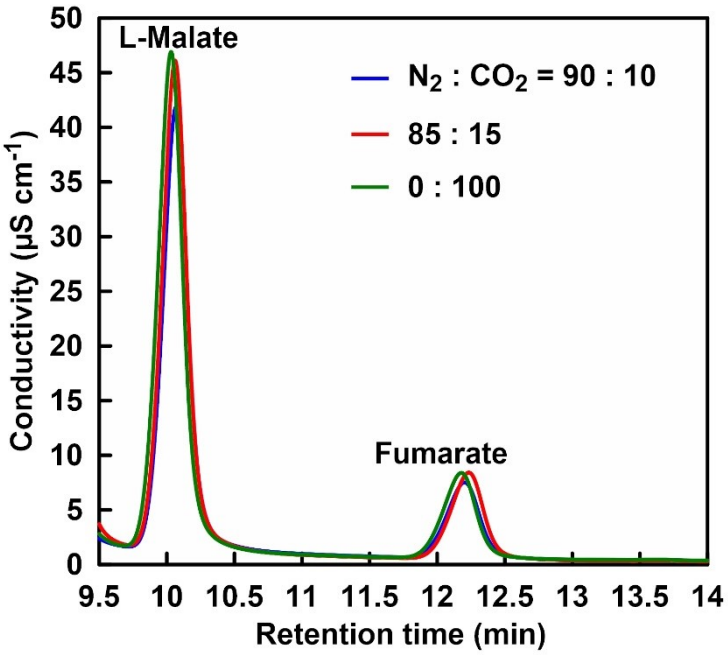

Figure S9. A chart of an ion chromatogram for L-malate concentration in the solution of sodium pyruvate (5.0 mM), manganese chloride (5.0 mM), ATP (5.0 mM), NADH (5.0 mM), acetyl-CoA (1.0 mM), PC (1.0 U), MDH (10 U) and FUM (0.5 U) in 5.0 mL of 500 mM HEPES buffer (pH 7.2) with a mixture gas of CO_2 and N_2 after 2 h incubation. The composition of the gas phase including ballon was adjusted to 90 % N_2 and 10 % CO_2, 85 % N_2 and 15 % CO_2 100% CO_2 gas.