

Lipid Oxidation

Materials:

- Erlenmeyer
- Magnetic Stirrer
- Hydrogene Peroxide (H_2O_2)
- Ferrous Sulfate (FeSO_4)
- Sulfuric Acid (H_2SO_4)

Hazards and Safety:

- Hydrogene Peroxide (H_2O_2) 35%



Hazard statement(s)

H302 Harmful if swallowed.

H315 Causes skin irritation.

H318 Causes serious eye damage.

H335 May cause respiratory irritation.

H412 Harmful to aquatic life with long lasting effects.

Precautionary statement(s)

P261 Avoid breathing mist or vapors.

P273 Avoid release to the environment.

P280 Wear protective gloves/ eye protection/ face protection.

P301 + P312 IF SWALLOWED: Call a POISON CENTER/ doctor if you feel unwell.

P302 + P352 IF ON SKIN: Wash with plenty of water.

P305 + P351 + P338 IF IN EYES: Rinse cautiously with water for several minutes.

Remove contact lenses, if present and easy to do. Continue rinsing.

Disposal: All solutions are of low concentrations. Dilution method and disposal under cold water in the canalization (ETH Zürich guideline)

- Ferrous Sulfate (FeSO_4)

**Hazard statement(s)**

H302 Harmful if swallowed.

H315 Causes skin irritation.

H319 Causes serious eye irritation.

Precautionary statement(s)

P264 Wash skin thoroughly after handling.

P270 Do not eat, drink or smoke when using this product.

P280 Wear protective gloves/ eye protection/ face protection.

P301 + P312 IF SWALLOWED: Call a POISON CENTER/ doctor if you feel unwell.

P302 + P352 IF ON SKIN: Wash with plenty of water.

P305 + P351 + P338 IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.

Disposal: Dilute high concentrations in water and dispose in inorganic acid container

- Sulfuric Acid (H_2SO_4)

**Hazard statement(s)**

H290 May be corrosive to metals.

H314 Causes severe skin burns and eye damage.

Precautionary statement(s)

P234 Keep only in original packaging.

P280 Wear protective gloves/ protective clothing/ eye protection/ face

protection.

P303 + P361 + P353 IF ON SKIN (or hair): Take off immediately all contaminated clothing. Rinse skin with water.

P304 + P340 + P310 IF INHALED: Remove person to fresh air and keep comfortable for breathing. Immediately call a POISON CENTER/ doctor.

P305 + P351 + P338 IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.

P363 Wash contaminated clothing before reuse.

Disposal: Inorganic acids (If to concentrated dilute first)

General Precaution:



Preparation of Solutions:

1) Lipid Mix 1000uM Solution

- MeOH:IPA (1:1) or Chloroform (CHCl_3)

2) Aqueous FeSO_4 Solution: 100mL

- 10mM Solution
- $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$: 0.278g
- Fill to 100mL milliQ water

$$\begin{aligned}n(\text{FeSO}_4) &= M(\text{FeSO}_4) \times V \\&= 10 \times 10^{-3} \text{mol.L}^{-1} \times 0.1\text{L} \\&= 1 \times 10^{-3} \text{mol} \\m(\text{FeSO}_4) &= \text{MW}(\text{FeSO}_4) \times n(\text{FeSO}_4) \\&= 151.908 \text{g.mol}^{-1} \times 1 \times 10^{-3} \text{mol} \\&= 0.1519 \text{g}\end{aligned}$$

$$\begin{aligned}
 m(\text{FeSO}_4 \bullet 7\text{H}_2\text{O}) &= m(\text{FeSO}_4) \times \text{MW}(\text{FeSO}_4 \bullet 7\text{H}_2\text{O}) / \text{MW}(\text{FeSO}_4) \\
 &= 0.1519\text{g} \times (278.01/151.908) \\
 &= 0.278\text{g}
 \end{aligned}$$

3) Hydrogene Peroxide Solution: 100mL

- 100mM Solution
- H₂O₂ 35% (w/w) Solution: 0.85 mL

Molarity of 35% solution:

$$\rho = 1.11\text{g.mL}^{-1}$$

$$\begin{aligned}
 \text{mass concentration}(\text{H}_2\text{O}_2) &= 0.35 \times 1.135\text{g.mL}^{-1} \\
 &= 0.39725\text{g.mL}^{-1}
 \end{aligned}$$

$$\begin{aligned}
 n(\text{H}_2\text{O}_2) \text{ in 1mL} &= m(\text{H}_2\text{O}_2) / \text{MW}(\text{H}_2\text{O}_2) \\
 &= 0.39725\text{g} / 34.0147 \text{ g.mol}^{-1} \\
 &= 0.01167\text{mol}
 \end{aligned}$$

$$\begin{aligned}
 \text{Molarity}(\text{H}_2\text{O}_2) &= n(\text{H}_2\text{O}_2) / V \\
 &= 0.01167\text{mol} / 1 \times 10^{-3}\text{L} \\
 &= 11.67 \text{ M}
 \end{aligned}$$

Volume of H₂O₂ 35% for 100mM solution:

$$\begin{aligned}
 V(\text{H}_2\text{O}_2 \text{ 30\%}) &= 0.1\text{mM} \times 100\text{mL} / 11.67\text{M} \\
 &= 0.85\text{mL}
 \end{aligned}$$

4) Sulfuric Acid Solution: 10mL

- 1M Solution
- H₂SO₄: 550 uL (97% solution)
- Fill to 10mL milliQ water

Molarity of 96% solution:

$$\rho = 1.84\text{g.mL}^{-1}$$

$$\begin{aligned}
 \text{mass concentration}(\text{H}_2\text{SO}_4) &= 0.96 \times 1.84\text{g.mL}^{-1} \\
 &= 1.76\text{g.mL}^{-1}
 \end{aligned}$$

$$\begin{aligned}
 n(\text{H}_2\text{SO}_4) \text{ in 1mL} &= m(\text{H}_2\text{SO}_4) / \text{MW}(\text{H}_2\text{SO}_4) \\
 &= 1.76\text{g} / 98.08 \text{ g.mol}^{-1} \\
 &= 0.0179\text{mol}
 \end{aligned}$$

$$\begin{aligned}
 \text{Molarity}(\text{H}_2\text{SO}_4) &= n(\text{H}_2\text{SO}_4) / V \\
 &= 0.0179\text{mol} / 1 \times 10^{-3}\text{L}
 \end{aligned}$$

$$= 17.9\text{M}$$

Volume of H_2SO_4 35% for 1M solution:

$$V(\text{H}_2\text{SO}_4 \text{ 96\%}) = 1\text{M} \times 10\text{mL} / 17.9\text{M}$$

$$= 0.55\text{mL}$$

Protocol 1:

Reagents:

FeSO_4 (2.5 mM) and H_2O_2 (50 mM) at 20°C

Procedure:

- Fenton Reagents in 1.5mL Eppendorf Tube:
 - H_2O_2 : 200 μL (100 mM)
 - FeSO_4 : 100 μL (10 mM)
- Add Sulfuric Acid to adjust pH 2-4
- Wait for the appearance of brown color (Fe^{3+})
- Add 100 μL Lipid mixture to the solution above.

End Reaction:

- Evaporate the solvents
- Add 200 μL MeOH:IPA + BHT (30 μM)

Protocol 2:

Reagents:

FeSO_4 (0.5 mM) and H_2O_2 (0.25 Mm) at 37°C

Solvent Preparation:

Aqueous FeSO_4 Solution: 100mL

- 2mM solution
- FeSO_4 (10mM): 20 mL
- milliQ Water: 80 mL

Hydrogene Peroxide Solution: 100mL

- 5mM
- H_2O_2 (100mM): 5mL
- milliQ water: 95mL

Hydrogene Peroxide Solution: 100mL

- 0.5mM
- H₂O₂ (5mM): 10mL
- milliQ water: 90mL

Procedure:

- Fenton Reagents in 1.5mL Eppendorf Tube:
 - H₂O₂: 200 uL (0.5 mM)
 - FeSO₄: 100 uL (2 mM)
- Add Sulfuric Acid to adjust pH 2-4
- Wait for the appearance of brown color (Fe³⁺)
- Add 100 uL Lipid mixture to the solution above.

End Reaction:

- Evaporate the solvents
- Add 200 uL MeOH:IPA + BHT (30 uM)