

Ferroptosis Pathway Guide



Ferroptosis is an iron-dependent cell death that is triggered when oxidized polyunsaturated fatty acids (PUFAs) in lipid membranes are not repaired or protected by glutathione peroxidase 4 (GPX4), ferroptosis suppressor protein 1 (FSP1), or tetrahydrobiopterin activity. Cayman offers a comprehensive tool kit to study this process.

Identify Novel Inducers of Ferroptosis

GPX4 Inhibitor Screening Assay Kit

Item No. 701880

- Includes recombinant human GPX4 and positive control GPX4 inhibitor ML-162
- Assay 45 samples in duplicate or 29 samples in triplicate
- Plate-based colorimetric measurement (340 nm)

FSP1 Fluorescent Inhibitor Screening Assay Kit

Item No. 701900

- Includes recombinant human FSP1 and positive control FSP1 inhibitor iFSP1
- Assay 45 samples in duplicate or 29 samples in triplicate
- Plate-based fluorometric measurement (590 nm)

GPX Detection

| Item No. | Product Name |
|----------|---|
| 703102 | Glutathione Peroxidase Assay Kit |
| 26906 | GPX4 (human, recombinant); selenocysteine incorporation confirmed by MS |
| 10005258 | GPX4 Polyclonal Antibody |

Glutathione (GSH) Activity Assays

| Item No. | Product Name |
|----------|--|
| 703002 | Glutathione Assay Kit |
| 600360 | Glutathione Cell-Based Detection Kit (Blue Fluorescence) |
| 703202 | Glutathione Reductase Assay Kit |

GSH Derivatives and Antibody

| Item No. | Product Name |
|----------|---|
| 14953 | Glutathione ethyl ester |
| 10007461 | L-Glutathione, reduced |
| 10010013 | Glutathione S-Transferase Polyclonal Antibody |

FSP1 Detection

| Item No. | Product Name |
|----------|----------------------------|
| 29611 | FSP1 (human, recombinant) |
| 29554 | Coumarin-Quinone Conjugate |

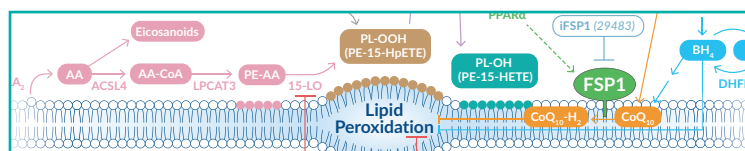
Lipoxygenases & Inhibitor Screening Assay

| Item No. | Product Name |
|----------|--|
| 60402 | 5-Lipoxygenase (human, recombinant) |
| 10341 | 12-Lipoxygenase (platelet-type, mouse recombinant) |
| 10011263 | 15-Lipoxygenase-2 (human recombinant) |
| 760700 | Lipoxygenase Inhibitor Screening Assay Kit |

Oxidized Phospholipids

| Item No. | Product Name |
|----------|--|
| 21138 | 1-Stearoyl-2-15(S)-HETE-sn-glycero-3-PC |
| 21139 | 1-Stearoyl-2-15(S)-HETE-sn-glycero-3-PE |
| 26531 | 1-Stearoyl-2-15(S)-HpETE-sn-glycero-3-PC |
| 25856 | 1-Stearoyl-2-15(S)-HpETE-sn-glycero-3-PE |

See all oxidized phospholipids at www.caymanchem.com



Learn about various ferroptosis mediators inside this guide.



Cayman products that induce Ferroptosis

Cayman products that inhibit Ferroptosis

GSH Availability

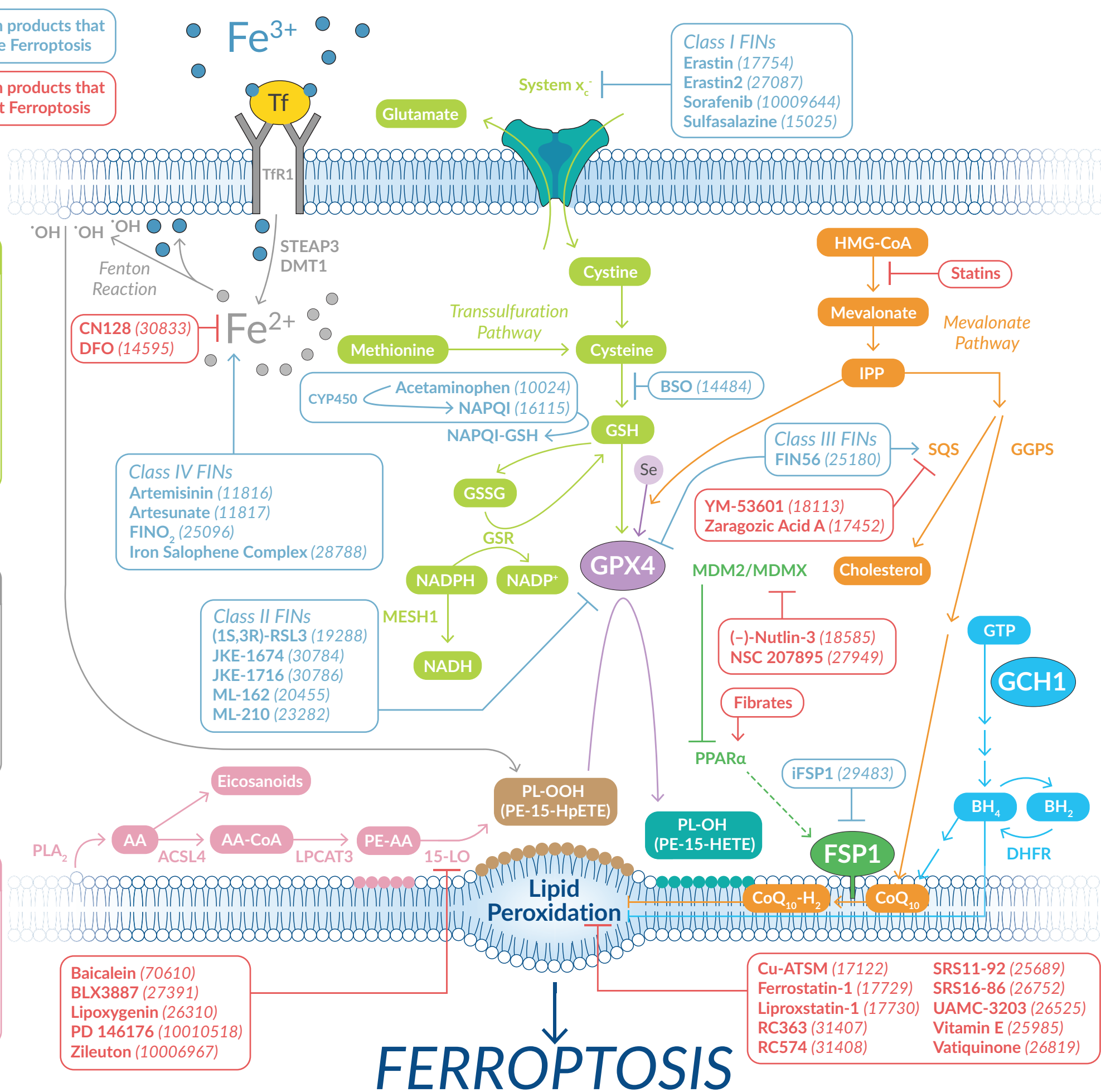
The cystine/glutamate exchanger regulates intracellular GSH content to balance cellular redox homeostasis. Extracellular cystine is transported into the cell and converted into cysteine for GSH synthesis. MESH1, an NADPH phosphatase, depletes NADPH needed to regenerate reduced GSH.

Free Radical Lipid Peroxidation

Iron is imported through the transferrin receptor, reduced by endosomal STEAP3, and released into the cytosol by DMT1. Through the Fenton reaction, excessive ferrous iron (Fe^{2+}) generates highly reactive hydroxyl radicals, which oxidize PUFAs.

Enzymatic Lipid Peroxidation

PUFAs such as AA are enriched in membrane phospholipids through the action of remodeling enzymes like PLA_2 , ACSL4, and LPCAT3. They can be oxidized by cellular lipoxygenases such as 15-LO.



Mevalonate Pathway

Isopentenyl pyrophosphate (IPP) enables insertion of selenocysteine into the catalytic center of GPX4, which is important for its antioxidant activity. Coenzyme Q₁₀ (CoQ₁₀), a substrate of FSP1, is also produced from this pathway.

GPX4

GPX4, a selenoprotein, catalyzes the reduction of lipid peroxides at the expense of GSH. When GPX4 activity is hindered, lipid peroxides accumulate and ultimately cause cell death.

FSP1

Myristoylated, membrane-bound FSP1 reduces CoQ₁₀ to CoQ₁₀-H₂, which halts lipid peroxidation. The MDM2/MDMX complex reduces expression of FSP1 by inhibiting PPARα activity.

GCH1

GTP cyclohydrolase 1 (GCH1) initiates the synthesis of BH₂ and BH₄, which inhibits ferroptosis by protecting a subset of phospholipids from peroxidation and also by increasing *de novo* synthesis of CoQ₁₀. BH₄ is regenerated by dihydrofolate reductase (DHFR).

FERROPTOSIS

Lipid ROS Detection

Cayman provides several convenient methods to detect the presence of lipid ROS. This includes assays for direct quantification of lipid hydroperoxides as well as assays that quantify end product reactive aldehydes, such as malondialdehyde or 4-hydroxy nonenal (4-HNE).

Lipid ROS Assay Kits

| Item No. | Product Name | Mode of Action |
|----------|--|--|
| 501140 | DHN-MA EIA Kit | Measures a metabolite of 4-HNE, a byproduct of lipid peroxidation |
| 705002 | Lipid Hydroperoxide (LPO) Assay Kit | Measures hydroperoxides directly using the redox reactions with ferrous ions |
| 601290 | ROS Detection Cell-Based Assay Kit (DHE) | Measures superoxide and hydrogen peroxide levels in living cells |
| 10009055 | TBARS Assay Kit | Measures malondialdehyde, a byproduct of lipid peroxidation |

Lipid ROS Probes

| Item No. | Product Name | Description |
|----------|--------------------------|--|
| 27086 | C11 BODIPY 581/591 | Lipid-soluble ratiometric fluorescent indicator of lipid oxidation |
| 62237 | DPPP | Fluorescent probe for detection of hydroperoxides |
| 13265 | 4-hydroxy Nonenal Alkyne | 4-HNE modified for click chemistry |

Lipid Peroxidation End Products

| Item No. | Product Name | Description |
|----------|---|---|
| 10004413 | 4-hydroperoxy 2-Nonenal | The hydroperoxide precursor of 4-HNE |
| 32100 | 4-hydroxy Nonenal | A lipid peroxidation product used as a marker of lipid peroxidation |
| 10185 | 4-oxo-2-Nonenal | A more recently identified product of lipid peroxidation |
| 10627 | 4-hydroxy Nonenal Glutathione (trifluoroacetate salt) | A major adduct formed by the reaction of 4-HNE with GSH; prevents the formation of DNA adducts by trapping of 4-HNE |
| 32110 | 4-hydroxy Nonenal Mercapturic Acid | A urinary metabolite of 4-HNE |

Lipophilic Antioxidants

| Item No. | Product Name | Description |
|----------|--|--|
| 89910 | BHT | A widely used synthetic antioxidant |
| 81880 | tetrahydro-L-Biopterin (hydrochloride) | A non-enzymatic redox sensitive antioxidant that limits lipid peroxide formation |
| 11506 | Coenzyme Q ₁₀ | A cofactor in the electron-transport chain whose reduced form acts as an antioxidant |
| 70530 | Ebselen | A glutathione peroxidase mimic |
| 15475 | Idebenone | A potent lipid antioxidant that prevents the generation of free radicals |
| 10008377 | α -Tocotrienol | A vitamin E analog |
| 10011659 | Trolox | A cell-permeable vitamin E derivative with antioxidant properties |

Oxidized Phospholipid Lipidomic Services

Cayman's scientific staff has decades of industry expertise in assay and methods development, sample preparation, and analysis. Our targeted panel of oxidized acyl chains (e.g., 20:4-OH, 20:4-OOH) will help you identify hydroperoxy and hydroxy lipids in your samples. A recent publication using our method reports that increased oxidized lipids in tumor cells leads to ferroptosis and with immunotherapy is linked to cancer cell death (*Nature* **569**, 270-274 (2019)).

Visit www.caymanchem.com/lipidomics to learn more.