

MitoProfile[®] Total OXPHOS + PDH + Controls ICC Antibody Kit

MS602a

Rev.1

DESCRIPTION

MitoProfile[®] OXPHOS/PDH Immunocytochemistry Protocol.

Kit Contents:

Monoclonal antibodies to detect assembly defects in the 5 OXPHOS complexes and PDH complex. They are of isotype IgG₁ or IgG_{2a}.

Antibodies for detecting OXPHOS complex/PDH complex assembly defects	
MS602-I	Complex I (NDUFB4)
MS602-II	Complex II 30 kD
MS602-III	Complex III Core 2
MS602-IV	Complex IV subunit 1
MS602-V	Complex V OSCP
MS602-P	PDH E1 α

Monoclonal antibodies for detecting the reference proteins are of IgG_{2b} isotype, allowing double labeling of cells to compare the per-cell level of each OXPHOS/PDH complex.

Antibodies for Reference proteins	
MS602-CV α	Complex V α
MS602-Porin	Porin

Secondary antibodies.

Fluorophore-conjugated secondary antibodies	
MS602-IgG1	IgG ₁ -FITC
MS602-IgG2a	IgG _{2a} -FITC
MS602-IgG2b	IgG _{2b} -TXRD

Storage:

Antibodies should be store at 4°C. The fluorophore-conjugated secondary antibodies should be stored away from light at 4°C.

ADDITIONAL MATERIALS REQUIRED

- Fibroblasts (or other adherent cells) which are ready to be seeded on 6-well tissue culture plates
- Tissue culture medium appropriate for the cells
- 6-well tissue culture plates (Fisher Scientific www.fishersci.com, cat# 07-200-80)
- Glass coverslips
- Broad-Tipped Forceps, 4 ½" long (Fisher Scientific www.fishersci.com, cat# 10-300)
- Coverglass staining jar (Electron Microscopy Services www.emsdiasum.com, cat# 72242-01)
- Microscope slides
- Paraformaldehyde (Electron Microscopy Services www.emsdiasum.com, cat# 15713)
- PBS, pH 7.4
 - 8 mM Na₂HPO₄
 - 1.4 mM KH₂PO₄
 - 140 mM NaCl
 - 2.7 mM KClAdjust pH to 7.4 with NaOH
- Antigen Retrieval Buffer
 - 100 mM Tris
 - 5% (w/v) ureaAdjust pH to 9.5 with HCl
- 0.1% (v/v) Triton X-100 in PBS
- Goat Serum (Invitrogen www.invitrogen.com, cat# 16210-064)
- DAPI nucleic acid stain, 1mg/mL
- Mounting medium
- Nail polish or glue (e.g. Duco Cement)

Equipment:

- Waterbath heated to 95°C
- Fluorescence microscope fitted with filters for:
 - Texas Red (TXRD) dye (Excitation λ 590 nm; Emission λ 620 nm).
 - Fluorescein isothiocyanate (FITC) dye (Excitation λ 488 nm; Emission λ 520 nm).
 - DAPI dye (Excitation λ 360 nm; Emission λ 460nm).

These filters can be purchased from Chroma Technology Corp (www.chroma.com) or Omega Optical (www.omegafilters.com).

CELL PREPARATION

The MS602a kit can be used for immunocytochemistry on patient-derived cultured fibroblasts or other adherent cells.

It is recommended that the cells are grown on 100-mm or 150-mm diameter tissue culture dishes and then seeded on glass coverslips in 6-well tissue culture plates before the day of the experiment. At least one 6-well tissue culture plate of cells is needed for testing a single cell line for OXPHOS/PDH assembly defects.

1. Sterilize glass coverslips by dipping them in 90% ethanol and carefully drying them over a flame for a few seconds.
2. Place each coverslip in sterile 6-well tissue culture plates (Figure 1).

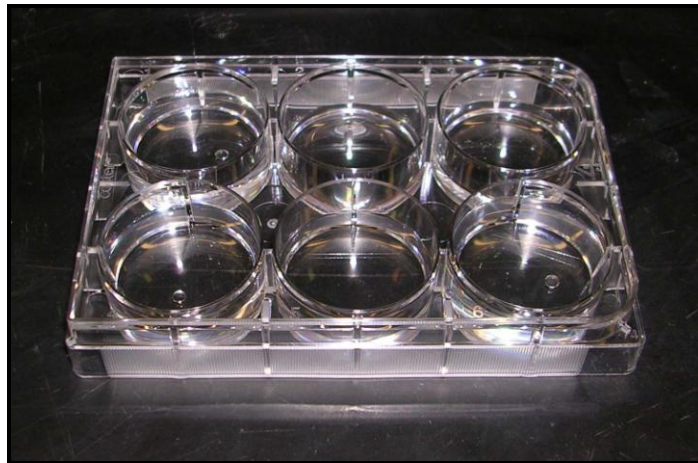


Figure 1. 6-well tissue culture plate.

3. Add 1-2 mL of cell suspension over each coverslip in the 6-well plates.
4. Grow the cells at 37°C in a humidified CO₂ incubator until they are 50-70% confluent.
Note: *The CO₂ level should be adjusted according to the type of medium used for growing the cells. Fibroblasts grown in High Glucose Dulbecco's Modified Eagle Medium with 10-20% fetal calf serum are maintained at pH 7.4 in a 10% CO₂ incubator.*
6. Aspirate the culture medium from each well.
7. Gently rinse the cells twice in PBS at room temperature. Do not let the cells dry out.
8. Fix the cells by incubating them in 4% (v/v) paraformaldehyde in PBS for 20 minutes at room temperature.
9. Rinse the cells three times with PBS.
10. The cells can be stored in 0.02% (w/v) sodium azide in PBS at 4°C for several days or be analyzed immediately using the MS602a kit.

MS602a PROCEDURE

1. Preheat the Antigen Retrieval Buffer (100 mM Tris, 5% (w/v) urea, pH 9.5) to 95°C. This can be done by heating the buffer in a coverglass staining jar which is placed in a waterbath at 95°C.
2. Using a small pair of broad-tipped forceps, place the coverslips carefully in the Antigen Retrieval Buffer in the coverglass staining jar, making note of which side of the coverslips the cells are on (Figure 2).
3. Heat at 95°C for 10 minutes.

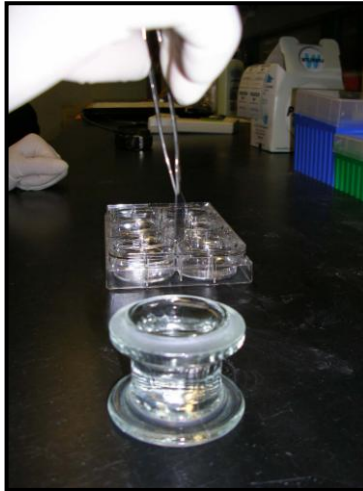


Figure 2. Coverglass staining jar.

4. Remove the coverslips from the Antigen Retrieval Buffer and immerse them, with the side containing the cells facing up, in PBS, in the 6-well tissue culture plates.
5. Rinse the cells 3 times in PBS.
6. Permeabilize the cells by incubating them in 0.1% Triton[®] X-100 in PBS for 10-15 minutes at room temperature.
7. Rinse the cells 3 times in PBS.
8. Incubate the cells in 10% goat serum in PBS for 1 hour at room temperature.

Incubate cells in primary antibodies:

9. Dilute the Porin antibody (IgG_{2b}) or Complex V α antibody (IgG_{2b}) to a final concentration of 0.5-2 μ g/mL in 10% goat serum. The final volume should be sufficient to cover each coverslip (0.5 -1 mL per coverslip).
10. Divide the diluted Porin antibody or Complex V α antibody into 6 equal aliquots.
11. Add the primary antibodies against Complex I-V, and PDH-E1 α to the aliquots; the concentrations mentioned in Table 1 are recommended when analyzing fibroblasts. *If using other adherent cells, the optimal dilutions need to be determined.*
12. Add aliquots 1- 6 to wells 1-6 of each 6-well tissue culture plate.

13. Incubate the cells in the 6 pairs of primary antibodies at 4°C, overnight, or at room temperature for 2 hours.

Aliquot/Well	OXPPOS / PDH antibody	Final Concentration (µg/mL)	Isotype
1	Complex I 15 kD	10 - 15	IgG ₁
2	Complex II 30 kD	5 - 10	IgG _{2a}
3	Complex III Core 2	0.25 - 1	IgG ₁
4	Complex IV subunit 1	5 - 10	IgG _{2a}
5	Complex V OSCP	0.5 - 2	IgG ₁
6	PDH-E1 α	5 - 15	IgG ₁

Table 1. Antibody concentrations.

14. Rinse the cells in 1% goat serum in PBS 3 times for 10 minutes.

Incubate the cells in pairs of secondary antibodies:

15. Dilute the three fluorophore-conjugated secondary antibodies, IgG₁-FITC, IgG_{2a}-FITC and IgG_{2b}-TXRD, in 10% goat serum (Table 2) and incubate the cells in them, away from light, for 2 hours.

Well	Secondary antibodies
1	2-4 µg/mL IgG ₁ -FITC and 2 µg/mL IgG _{2b} TXRD
2,4	2 µg/mL IgG _{2a} -FITC and 2 µg/mL IgG _{2b} -TXRD
3,5,6	2 µg/mL IgG ₁ -FITC and 2 µg/mL IgG _{2b} -TXRD

Table 2. Secondary antibodies.

16. Rinse cells in 1% goat serum 3 times for 10 minutes, away from light.
17. Dilute the DAPI stain to a final concentration of 300 ng/mL in 1% goat serum.
18. Incubate the cells for 10 minutes in the diluted DAPI.
19. Label a microscope slide for each coverslip.
20. Add a drop of mounting medium to each slide.
21. Pick up each coverslip with a forceps and place it on the mounting medium, with the cell-side face down.
22. Apply nail polish or glue along the edges of the coverslips to seal them to the slides.
23. Visualize the cells using a fluorescence microscope equipped with a Texas red filter, a Fluorescein isothiocyanate filter and a DAPI filter.