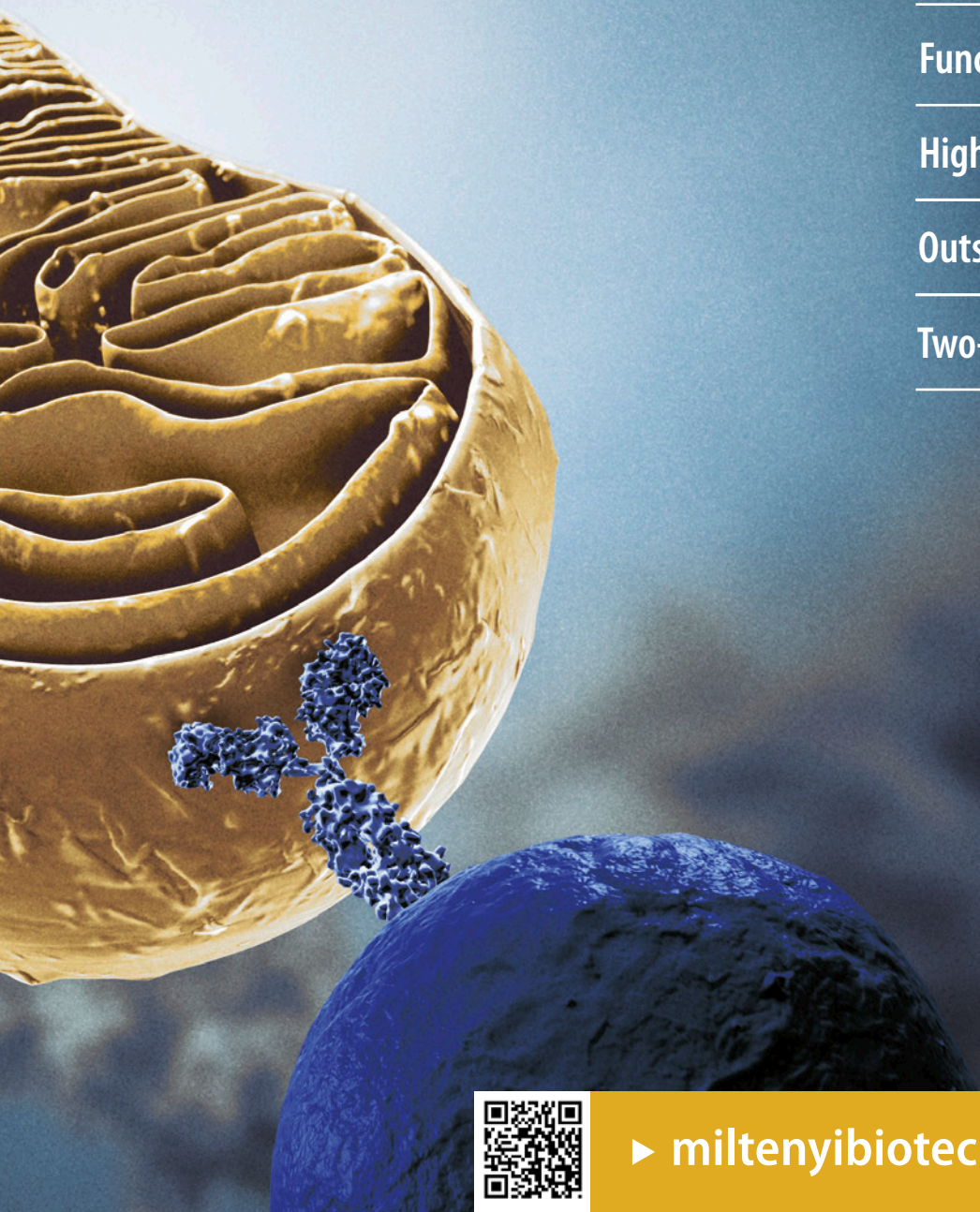
Miltenyi Biotec

MACS® Technology

Magnetic mitochondria isolation



- Functional mitochondria
- High purity
- Outstanding yield
- Two-hour workflow

▶ miltenyibiotec.com/mitochondria

Mitochondria Isolation Kit, human

For 25 isolations from human tissue or cells

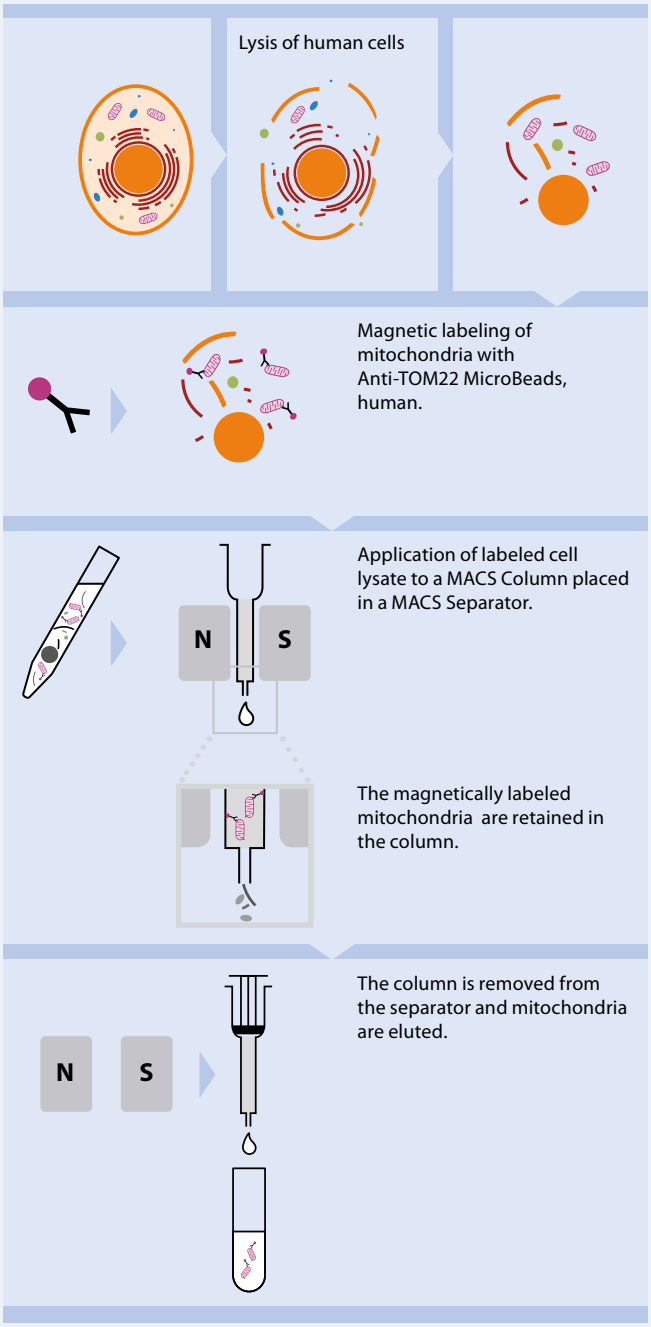


Figure 1: Principle of MACS Technology for mitochondria isolation

The **Mitochondria Isolation Kit, human** enables isolation of intact, viable mitochondria from human cells or tissue in less than two hours. The kit's protocol is based on the renowned MACS® Technology that combines high yield with purity and integrity of the isolated mitochondria (figs. 2 and 3). After cell lysis, the translocase of the outer mitochondrial membrane 22 (TOM22) is targeted by Anti-TOM22 MicroBeads, human to enable magnetic isolation of functional human mitochondria. See figure 1 for details.

Functional mitochondria preparation

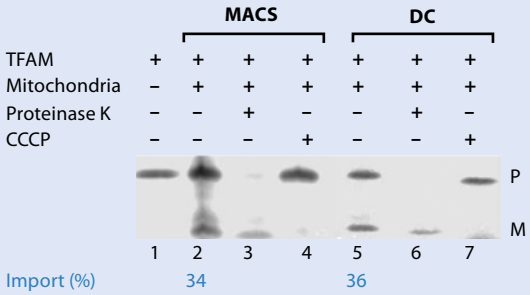


Figure 2: Mitochondria were prepared using the Mitochondria Isolation Kit, human (MACS) or by differential centrifugation (DC) and were then incubated for 1 h with ^{35}S-labeled TFAM. Import was studied by SDS-PAGE and fluoro-graphy. Lane 1: Control lysate of *in vitro* translated TFAM. Lane 2–7: Proteins from sedimented mitochondria. P= Precursor protein. M= Imported mature protein. The data suggest that the function of mitochondria preparations isolated with the Mitochondria Isolation Kit, human is fully maintained. (Courtesy of Dr. H.-T. Hornig-Do, Newcastle, UK)

Purest mitochondria preparation

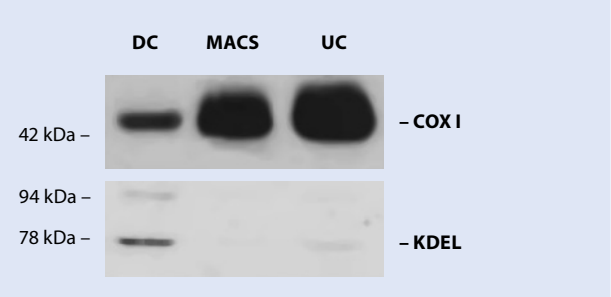


Figure 3: Mitochondria were prepared by different protocols. Amounts of COX I (mitochondria) and KDEL (endoplasmic reticulum) in the mitochondrial fractions obtained using differential centrifugation (DC), Mitochondria Isolation Kit, human (MACS), and ultracentrifugation (UC) were analyzed by Western blotting. Compared to DC and UC, mitochondria isolated with the Mitochondria Isolation Kit, human have no detectable contamination with ER. (Courtesy of Dr. H.-T. Hornig-Do, Newcastle, UK)

Mitochondria Isolation Kit, mouse tissue

For 25 isolations from mouse tissue

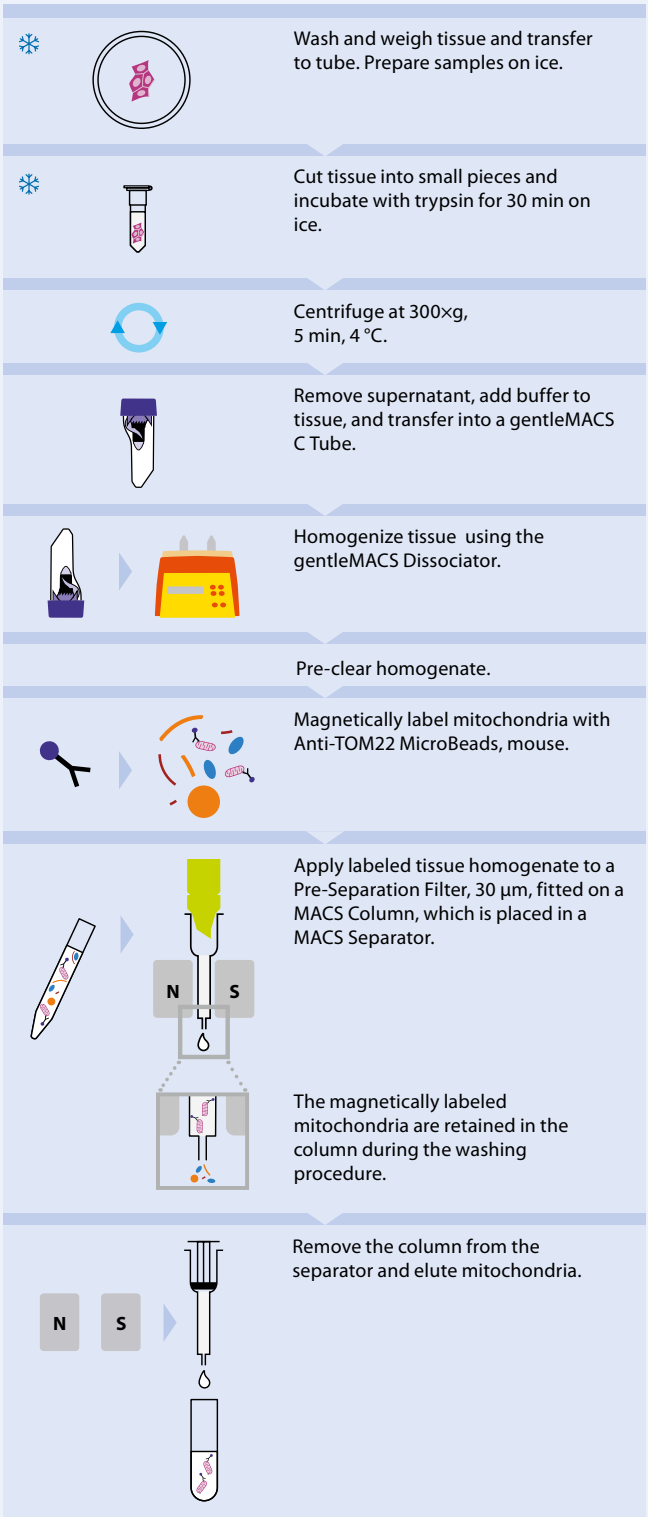


Figure 4: Integrated tissue dissociation followed by mitochondria isolation.

The **Mitochondria Isolation Kit, mouse tissue** is intended for isolation of mitochondria from any mouse tissue such as brain, liver, or skeletal muscle. Tissues can be dissociated with virtually any protocol although the use of the gentleMACS™ Dissociator is preferred because this will save time and ensures standardization of tissue dissociation and reproducible results (see fig. 4). Similar to the Mitochondria Isolation Kit, human, this kit allows for enrichment of functional and pure mitochondria (figs. 5 and 6).

Enrichment of functional mitochondria

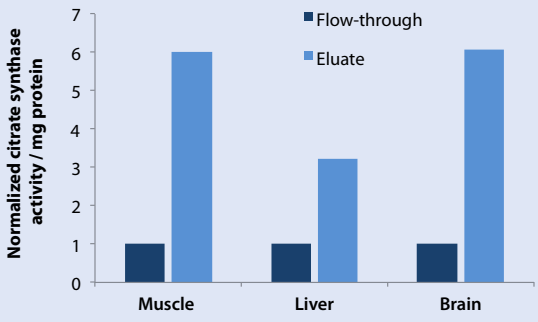


Figure 5: Mitochondria were prepared from various mouse tissues using the Mitochondria Isolation Kit, mouse tissue. The mitochondria preparations from muscle, liver and brain as well as the flow-through fractions were analyzed by a coupled enzymatic citrate synthase assay. The data indicate that citrate synthase activity, normalized to the activity per mg protein assayed in the flow-through fraction, is highly enriched in the eluate.

High purity liver mitochondria

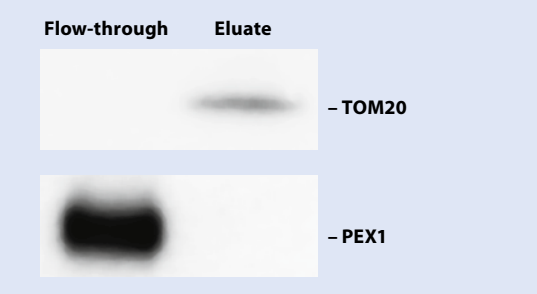


Figure 6: After isolation from liver using the Mitochondria Isolation Kit, mouse tissue, equal amounts of mitochondrial protein lysates were analyzed by Western blotting against TOM20. PEX1, a cytoplasmic protein anchored to peroxisomal membranes, served as a control. The results show an effective enrichment of mitochondria and no contamination of the mitochondria fraction by peroxisomes.

Product overview

Product	Capacity	Components	Order no.
Mitochondria isolation from human tissues or cells			
Mitochondria Isolation Kit, human	25 isolations	1.25 mL Anti-TOM22 MicroBeads, human, buffers, LS Columns	**130-094-532**
Mitochondria MidiMACS Starting Kit, human	25 isolations	1 Mitochondria Isolation Kit, 1 MidiMACS™ Separator, 1 MACS MultiStand	**130-094-872**
Mitochondria QuadroMACS Starting Kit, human	25 isolations	1 Mitochondria Isolation Kit, 1 QuadroMACS™ Separator, 1 MACS MultiStand	**130-094-833**
Mitochondria isolation from mouse tissue			
Mitochondria Isolation Kit, mouse tissue	25 isolations	1.25 mL Anti-TOM22 MicroBeads, mouse, buffers, LS Columns, Pre-Separation Filters, 30 µm	**130-096-946**
Mitochondria MidiMACS Starting Kit, mouse tissue	25 isolations	Mitochondria Isolation Kit, MidiMACS Separator, 1 MACS MultiStand	**130-097-039**
Mitochondria QuadroMACS Starting Kit, mouse tissue	25 isolations	Mitochondria Isolation Kit, QuadroMACS Separator, 1 MACS MultiStand	**130-097-040**
Related products			
gentleMACS Dissociator	1–2 samples in parallel	Instrument, 25 C Tubes, 25 M Tubes, power cord, user manual	**130-093-235**
gentleMACS Octo Dissociator	up to 8 samples in parallel	Instrument, power cord, user manual	**130-095-937**
Neural Tissue Dissociation Kit (P)	50 digestions	2.5 mL Solution 1, 2×50 mL Solution 2, 1.5 mL Solution 3, 1 vial Solution 4, 1 mL Storage Buffer	**130-092-628**
Neural Tissue Dissociation Kit (T)	50 digestions	10 mL Solution 1, 2×50 mL Solution 2, 1.5 mL Solution 3, 1 vial Solution 4, 1 mL Storage Buffer	**130-093-231**
Tumor Dissociation Kit, human	25 digestions	2 vials Solution 1, 1 vial Solution 2, 1 vial Solution 3, 1 mL Reconstitution Buffer	**130-095-929**
Tumor Dissociation Kit, mouse	50 digestions	2 vials Solution 1, 1 vial Solution 2, 1 vial Solution 3, 1 mL Reconstitution Buffer	**130-096-730**
Brain Tumor Dissociation Kit (P), human	25 digestions	1.25 mL Solution 1, 2×50 mL Solution 2, 1.5 mL Solution 3, 1 vial Solution 4, 1 mL Storage Buffer	**130-095-942**
Brain Tumor Dissociation Kit (T), human	25 digestions	4 mL Solution 1, 2×50 mL Solution 2, 1.5 mL Solution 3, 1 vial Solution 4, 1 mL Storage Buffer	**130-095-939**

Testimonials and References

"Well-coupled mitochondria are a prerequisite to achieving reliable reproducible results in nearly all functional assays…mitochondria isolated with the MACS protocol were found to be better coupled than those obtained using DC…"

Hornig-Do, H.T. *et al.* (2009) Anal. Biochem. 389: 1–5.

"In order to maximize mitochondria enrichment efficiency and minimize contaminations from other organelles, we employed a newly developed method for mitochondria isolation based on superpara-magnetic microbeads conjugated to anti-TOM22 antibody. The protocol is fast, reproducible, and standardized, resulting in mitochondria of high purity, with minimal contamination from cytoskeleton, cytosol, Golgi apparatus, endosome, endoplasmic reticulum, and nucleus."

Guo, T. *et al.* (2010) Mol. Cell. Proteomics 9: 2629–2641.

References

Hornig-Do, H.T. *et al.* (2009) Isolation of functional pure mitochondria by superparamagnetic microbeads. Anal. Biochem. 389: 1–5.

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Minet, A.D. and Gaster, M. (2010) ATP synthesis is impaired in isolated mitochondria from myotubes established from type 2 diabetic subjects. Biochem. Biophys. Res. Comm. 402: 70–74.

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Barrey, E. *et al.* (2011) Pre-microRNA and mature microRNA in human mitochondria. PLoS ONE 6: e20220.

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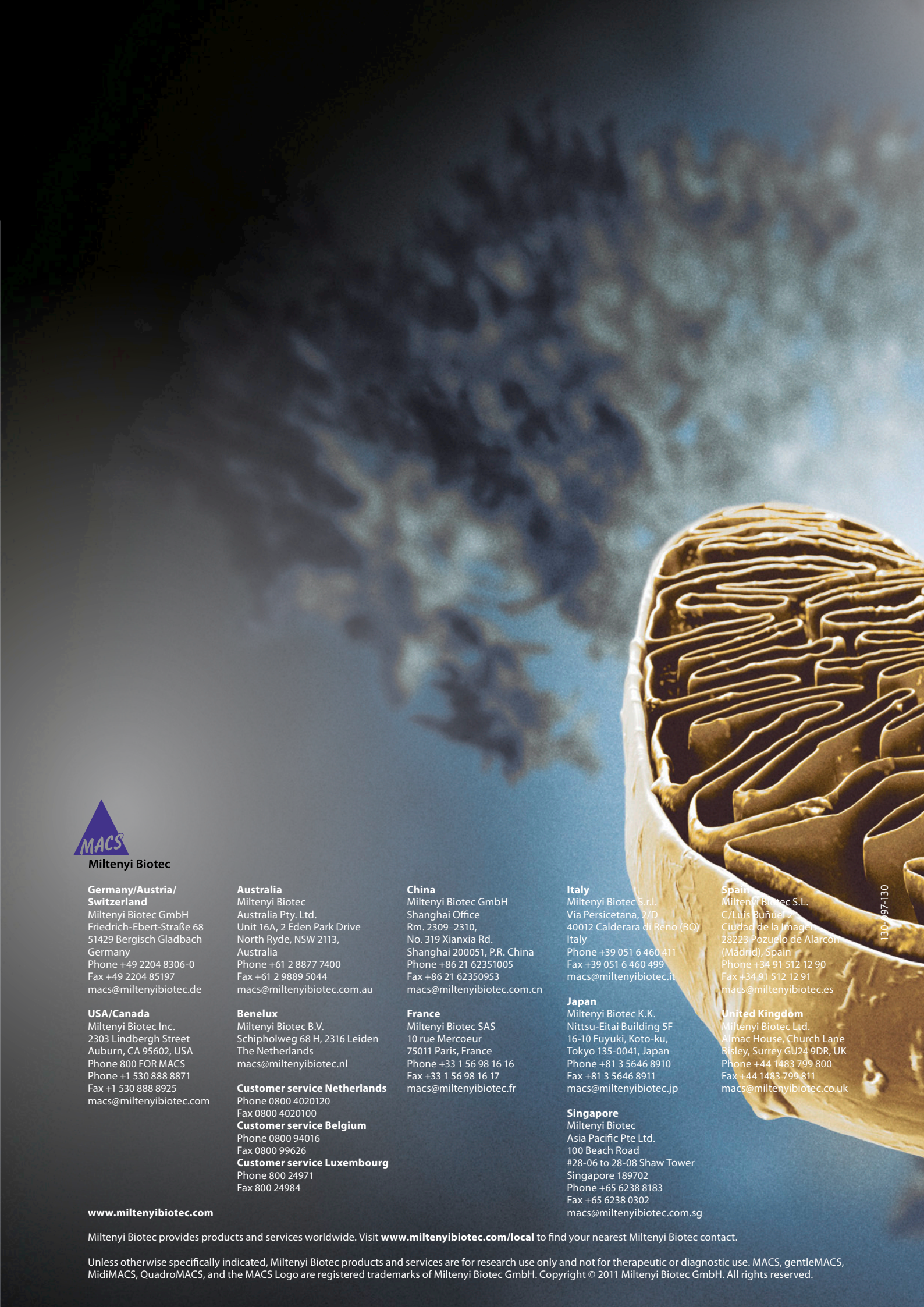

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