

OptiPrep™ Reference List RS02

Purification and analysis of peroxisomes

There are several OptiPrep™ Application Sheets that are relevant to the isolation and analysis of peroxisomes using iodixanol gradients and three types of gradient have been used:

Continuous iodixanol gradients: principally for the purification of peroxisomes from mammalian liver, but they have also been used for organelles from fungi, mainly yeast and also from plants.

Discontinuous iodixanol gradients: for mammalian liver and kidney, some marine organisms and plants.

Self-generated iodixanol gradients: for mammalian liver and cultured cells

- ◆ **Application Sheet S11** describes the use of a pre-formed continuous iodixanol gradient
- ◆ **Application Sheet S12** describes the use of a discontinuous iodixanol gradient
- ◆ **Application Sheet S13** describes the use of a self-generated iodixanol gradient
- ◆ **Application Sheet S57** describes the use of a continuous iodixanol gradient for yeast peroxisomes

In addition there are two Application Sheets that are devoted to the analysis of the light mitochondrial fraction (LMF) and although these are not devoted specifically to peroxisomes, the latter are analyzed as part of a more general analysis of the LMF organelles, which include mitochondria, lysosomes and sometimes Golgi in addition to peroxisomes.

- ◆ **Application Sheet S15** describes the use of pre-formed continuous gradient
- ◆ **Application Sheet S16** describes the use of self-generated gradient

The bibliography below is divided into **gradient type, then tissue or cell source**. References are listed alphabetically according to **first author** and then, if required, chronologically. To aid identification of research topics, these are **highlighted in blue**.

1. Continuous gradients

1a. Brain (rodent)

Nawrotzki, R., Islinger, M., Vogel, I., Völkl, A. and Kirsch, J. (2012) *Expression and subcellular distribution of gephyrin in non-neuronal tissues and cells* Histochem. Cell. Biol., **137**, 471–482

1b. Fat pad (mammary)

Vapola, M.H., Rokka, A., Sormunen, R.T., Alhonen, L., Schmitz W., Conzelmann, E., Wärrä, A., Grunau, S., Antonenkov, V.D. and Hiltunen, J.K. (2014) *Peroxisomal membrane channel Pxm2 in the mammary fat pad is essential for stromal lipid homeostasis and for development of mammary gland epithelium in mice* Dev. Biol., **391**, 66–80

1c. Fibroblasts

Wiesinger, C., Kunze, M., Regelsberger, G., Forss-Petter, S. and Berger, J. (2013) *Impaired very long-chain Acyl-CoA β -oxidation in human X-linked adrenoleukodystrophy fibroblasts is a direct consequence of ABCD1 transporter dysfunction* J. Biol. Chem., **288**, 19269-19279

1d. Fungi

1d-1 *Paracoccidioides brasiliensis*

Brito, W.deA., Rezende, T.C.V., Parente, A.F., Ricart, C.A.O., de Sousa, M.V., Bão, N. and Soares, C.M.deA. (2011) *Identification, characterization and regulation studies of the aconitase of Paracoccidioides brasiliensis* Fungal Biol., **115**, 697-707

1d-2 Yeast

- Antonenkov, V.D.**, Mindthoff, S., Grunau, S., Erdmann, R. and Hiltunen, J.K. (2009) *An involvement of yeast peroxisomal channels in transmembrane transfer of glyoxylate cycle intermediates* Int., J. Biochem. Cell Biol., **41**, 2546–2554
- Cramer, J.**, Effelsberg, D., Girzalsky, W. and Erdmann, R. (2015) *Isolation of peroxisomes from yeast* Cold Spring Harb. Protoc; doi:10.1101/pdb.top074500
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- Debelyy, M.O.**, Platta, H.W., Saffian, D., Hensel, A., Thoms, S., Meyer, H.E., Warscheid, B., Girzalsky, W. and Erdmann, R. (2011) *Ubp15p, a ubiquitin hydrolase associated with the peroxisomal export machinery* J. Biol. Chem., **286**, 28223–28234
- Effelsberg, D.**, Cruz-Zaragoza, L.D., Tonillo, J., Schliebs, W. and Erdmann, R. (2015) *Role of Pex21p for piggyback import of Gpd1p and Pnc1p into peroxisomes of Saccharomyces cerevisiae* J. Biol. Chem., **290**, 25333–25342
- Effelsberg, D.**, Cruz-Zaragoza, L.D., Schliebs, W. and Erdmann, R. (2016) *Pex9p is a new yeast peroxisomal import receptor for PTS1-containing proteins* J. Cell Sci., **129**, 4057–4066
- Einwächter, H.**, Sowinski, S., Kunau, W-H. and Schliebs, W. (2001) *Yarrowia lipolytica Pex20p, Saccharomyces cerevisiae Pex18p/Pex 21p and mammalian Pex5pL fulfil a common function in the early steps of the peroxisomal PTS2 import pathway* EMBO Rep., **2**, 1035–1039
- Grunau, S.**, Mindthoff, S., Rottensteiner, H., Sormunen, R.T., Hiltunen, J.K., Erdmann, R. and Antonenkov, V.D. (2009) *Channel-forming activities of peroxisomal membrane proteins from the yeast Saccharomyces cerevisiae* FEBS J., **276**, 1698–1708
- Grunau, S.**, Lay, D., Mindthoff, S., Platta, H.W., Girzalsky, W., Just, W.W. and Erdmann, R. (2011) *The phosphoinositide 3-kinase Vps34p is required for pexophagy in Saccharomyces cerevisiae* Biochem. J. **434**, 161–170
- Kerssen, D.**, Hambruch, E., Klaas, W., Platta, H.W., de Kruijff, B., Erdmann, R., Kunau, W-H. and Schliebs, W. *Membrane association of the cycling peroxisome import receptor Pex5p* J. Biol. Chem., **281**, 27003–27015
- Mindthoff, S.**, Grunau, S., Steinfort, L.L., Girzalsky, W., Hiltunen, J.K., Erdmann, R. and Antonenkov, V.D. (2016) *Peroxisomal Pex11 is a pore-forming protein homologous to TRPM channels* Biochim. Biophys. Acta, **1863**, 271–283
- Oeljeklaus, S.**, Reinartz, B.S., Wolf, J., Wiese, S., Tonillo, J., Podwojski, K., Kuhlmann, K., Stephan, C. et al (2012) *Identification of core components and transient interactors of the peroxisomal importomer by dual-track stable isotope labeling with amino acids in cell culture analysis* J. Proteome Res. 2012, **11**, 2567–2580
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- Schäfer, A.**, Kerssen, D., Veenhuis, M., Kunau, W-H. and Schliebs, W. (2004) *Functional similarity between the peroxisomal PTS2 receptor binding protein Pex18p and the N-terminal half of the PTS1 receptor Pex5p* Mol. Cell Biol., **24**, 8895–8906
- Thoms, S.**, Debelyy, M.O., Nau, K., Meyer, H.E. and Erdmann, R. (2008) *Lpx1p is a peroxisomal lipase required for normal peroxisome morphology* FEBS J., **275**, 504–514
- Welker, S.**, Rudolph, B., Frenzel, E., Hagn, F., Liebis, G., Schmitz, G., Scheuring, J., Kerth, A., Blume, A. et al (2010) *Hsp12 is an intrinsically unstructured stress protein that folds upon membrane association and modulates membrane function* Mol. Cell, **39**, 507–520

1e HEK cells

- Okumoto, K.**, Ono, T., Toyama, R., Shimomura, A., Nagata, A. and Fujiki, Y. (2018) *New splicing variants of mitochondrial Rho GTPase-1 (Miro1) transport peroxisomes* J. Cell Biol., **217**, 619–633

1f HeLa cells

- Abe, S.**, Nagai, T., Masukawa, M., Okumoto, K., Homma, Y., Fujiki, Y. and Mizuno, K. (2017) *Localization of protein kinase NDR2 to peroxisomes and its role in ciliogenesis* J. Biol. Chem., **292**, 4089–4098
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1g. Liver (rodent)

- Antonenkov, V.D.**, Sormunen, R.T. and Hiltunen, J.K. (2004) *The behavior of peroxisomes in vitro: mammalian peroxisomes are osmotically sensitive particles* Am. J. Physiol., **287**, C1623-C16350
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- Antonenkov, V.D.**, Sormunen, R.T., Ohlmeier, S., Amery, L., Franssen, M., Mannaerts, G.P. and Hiltunen, J.K. (2006) *Localization of a portion of the liver isoform of fatty-acid-binding protein (L-FABP) to peroxisomes* Biochem. J., **394**, 475-484
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- Gijsbers, S.**, Van der Hoeven, G. and Van Veldhoven, P.P. (2001) *Subcellular study of sphingoid base phosphorylation in rat tissues: evidence for multiple sphingosine kinases* Biochim. Biophys. Acta, **1532**, 37-50
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Westin, M.A.K., Hunt, M.C. and Alexson, S.E.H. (2007) *Peroxisomes contain a specific phytanoyl-CoA/Pristanoyl-CoA thioesterase acting as a novel auxiliary enzyme in α - and β -oxidation of methyl-branched fatty acids in mouse* J. Biol. Chem., **282**, 26707-26716

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1h. Mammary fat pad

Vapola, M.H., Rokka, A., Sormunen, R.T., Alhonen, L., Schmitz W., Conzelmann, E., Wärrri, A., Grunau, S., Antonenkov, V.D. and Hiltunen, J.K. (2014) *Peroxisomal membrane channel Pxmp2 in the mammary fat pad is essential for stromal lipid homeostasis and for development of mammary gland epithelium in mice* Dev. Biol., **391**, 66–80

1i. Plant tissues

Arai, Y., Hayashi, M. and Nishimura, M. (2008) *Proteomic analysis of highly purified peroxisomes from etiolated Soybean cotyledons* Plant Cell Physiol., **49**, 526-539

Hossain, Z. and Komatsu, S. (2014) *Soybean proteomics* In Plant Proteomics: Methods Mol. Biol., **1072** (ed. Jorin-Novotny, J.V. et al), Springer Science+Business Media, LLC, pp 315-331

Komatsu, S. and Ahsan, N. (2009) *Soybean proteomics and its application to functional analysis* J. Proteom., **72**, 325-336

Palma, J.M., Corpas, F.J. and del Rio, L.A. (2009) *Proteome of plant peroxisomes: new perspectives on the role of these organelles in cell biology* Proteomics, **9**, 2301-2312

Reumann, S. (2011) *Toward a definition of the complete proteome of plant peroxisomes: Where experimental proteomics must be complemented by bioinformatics* Proteomics **11**, 1764–1779

1j. Retinal pigment cells

Abe, S., Nagai, T., Masukawa, M., Okumoto, K., Homma, Y., Fujiki, Y. and Mizuno, K. (2017) *Localization of protein kinase NDR2 to peroxisomes and its role in ciliogenesis* J. Biol. Chem., **292**, 4089–4098

1k. Review

Antonenkov, V.D. and Hiltunen, J.K. (2006) *Peroxisomal membrane permeability and solute transfer* Biochim. Biophys. Acta, Mol. Cell Res., **1763**, 1697-1706

2. Discontinuous gradients

2a. HEK cells

Ge, L., Melville, D., Zhang, M. and Schekman, R. (2013) *The ER–Golgi intermediate compartment is a key membrane source for the LC3 lipidation step of autophagosome biogenesis* eLife, **2**: e00947

Zhang, J., Kim, J., Alexander, A., Cai, S., Tripathi, D.N., Dere, R., Tee, A.R., Tait-Mulder, J., Di Nardo, A., Han, J.M., Kwiatkowski, E., Dunlop, E.A., Dodd, K.M., Folkert, R.D., Faust, P.L., Kastan, M.B., Sahin, M. and Walker, C.L. (2013) *A tuberous sclerosis complex signalling node at the peroxisome regulates mTORC1 and autophagy in response to ROS* Nat. Cell Biol., **15**, 1186-1196

2b. HeLa cells

Luo, J., Liao, Y-C., Xiao, J. and Song, B-L. (2017) *Measurement of cholesterol transfer from lysosome to peroxisome using an in vitro reconstitution assay* In Cholesterol Homeostasis; Methods and Protocols: Methods Mol. Biol., **1583** (ed. Gelissen, I.C. and Brown, A.J.), Springer Science+Business Media LLC, pp 141-161

Xiao, J., Luo, J., Hu, A., Xiao, T., Li, M., Kong, Z., Jiang, L., Zhou, Z., Liao, Y. et al (2019) *Cholesterol transport through the peroxisome-ER membrane contacts tethered by PI(4,5)P2 and extended synapto-tagmins* Sci. China Life. Sci., **62**, 1117-1135

2c. Hep-G2 cells

Chen, X-F., Tian, M-X., Sun, R-Q., Zhang, M-L., Zhou, L-S., Jin, L., Chen, L-L., Zhou, W-J. et al (2018) *SIRT5 inhibits peroxisomal ACOX1 to prevent oxidative damage and is down-regulated in liver cancer* EMBO Rep., **19**: e45124

Wang, W., Xia, Z-J., Farré, J-C. and Subramani, S. (2017) *TRIM37, a novel E3 ligase for PEX5-mediated peroxisomal matrix protein import* J. Cell Biol., **216**, 2843–2858

2d. Human fibroblasts

Beltran, P.M.J., Mathias, R.A. and Cristea, I.M. (2016) *A portrait of the human organelle proteome in space and time during cytomegalovirus infection* Cell Systems **3**, 361–373

2e. Kidney (rodent)

Mi, J., Garcia-Arcos, I., Alvarez, R., and Cristobal, S. (2007) *Age-related subproteomic analysis of mouse liver and kidney peroxisomes* Proteome Sci., **5**:19

Mi, J., Kirchner, E. and Cristobal, S. (2007) *Quantitative proteomic comparison of mouse peroxisomes from liver and kidney* Proteomics, **7**, 1916-1928

2f. Liver (chick embryo)

Labitzke, E.M., Diani-Moore, S. and Rifkind, A.B. (2007) *Mitochondrial P450-dependent arachidonic acid metabolism by TCDD-induced hepatic CYP1A5; conversion of EETs to DHETs by mitochondrial soluble epoxide hydrolase* Arch. Biochem. Biophys., **468**, 70-81

2g. Liver (rodent)

Amelina, H., Sjödin, M.O.D., Bergquist, J. and Cristobal, S. (2011) *Quantitative subproteomic analysis of age-related changes in mouse liver peroxisomes by iTRAQ LC-MS/MS* J.Chromatogr. B, **879**, 3393– 3400

Grant, P., Ahlemeyer, B., Karnati, S., Berg, T., Stelzig, I., Nenicu, A., Kuchelmeister, K., Crane, D.I. and Baumgart-Vogt, E. (2013) *The biogenesis protein PEX14 is an optimal marker for the identification and localization of peroxisomes in different cell types, tissues, and species in morphological studies* Histochem. Cell. Biol., **140**, 423–442

Karnati, S., Lüers, G., Pfreimer, S. and Baumgart-Vogt, E. (2013) *Mammalian SOD2 is exclusively located in mitochondria and not present in peroxisomes* Histochem. Cell Biol., **140**, 105–117

Lamhonwah, A-M., Skaug, J., Scherer, S. and Tein, I. (2003) *A third human carnitine/organic cation transporter (OCTN3) as a candidate for the 5q31 Crohn's disease locus (IBD5)* Biochem. Biophys. Res. Commun., **301**, 98-101

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Mi, J., Kirchner, E. and Cristobal, S. (2007) *Quantitative proteomic comparison of mouse peroxisomes from liver and kidney* Proteomics, **7**, 1916-1928

Salvi, M., Battaglia, V., Brunati, A.M., La Rocca, N., Tibaldi, E., Pietrangeli, P., Marcocci, L., Mondovi, B., Rossi, C.A. and Toninello, A. (2007) *Catalase takes part in rat liver mitochondria oxidative stress defense* J. Biol. Chem., **282**, 24407-24415

Spinazzola, A., Viscomi, C., Fernandez-Vizarrá, E., Carrara, F., D'Adamo, P., Calvo, S., Marsano, R.M., Donnini, C., Weiher, H., Strisciuglio, P., Parini, R., Sarzi, E., Chan, A., DiMauro, S., Rotig, A., Gasparini, P., Ferrero, I., Mootha, V.K., Tiranti, V. and Zeviani, M. (2006) *MPV17 encodes an inner mitochondrial membrane protein and is mutated in infantile hepatic mitochondrial DNA depletion* Nat. Genet., **38**, 570-575

Weng, H., Ji, X., Naito, Y., Endo, K., Ma, X., Takahashi, R., Shen, C., Hirokawa, G., Fukushima, Y. and Iwai, N. (2013) *Pex11 α deficiency impairs peroxisome elongation and division and contributes to nonalcoholic fatty liver in mice* Am. J. Physiol. Endocrinol. Metab., **304**, E187–E196

2h. Mouse embryo fibroblasts

Zhang, J., Kim, J., Alexander, A., Cai, S., Tripathi, D.N., Dere, R., Tee, A.R., Tait-Mulder, J., Di Nardo, A., Han, J.M., Kwiatkowski, E., Dunlop, E.A., Dodd, K.M., Folkert, R.D., Faust, P.L., Kastan, M.B., Sahin, M. and Walker, C.L. (2013) *A tuberous sclerosis complex signalling node at the peroxisome regulates mTORC1 and autophagy in response to ROS* Nat. Cell Biol., **15**, 1186-1196

2i. Mussels

Apraiz, I., Mi, J. and Cristobal, S. (2006) *Identification of proteomic signatures of exposure to marine pollutants in mussels (Mytilus edulis)* Mol. Cell. Proteom., **5**, 1274-1285

Apraiz, I., Cajaraville, M.P. and Cristobal, S. (2009) *Peroxisomal proteomics: Biomonitoring in mussels after the Prestige's oil spill* Mar. Pollut. Bull., **58**, 1815–1826

Cristobal, S. (2007) *Proteomics-based method for risk assessment of peroxisome proliferating pollutants in the marine environment* Methods Mol. Biol., **410**, 123-135

Mi, J., Orbea, A., Syme, N., Ahmed, M., Cajaraville, M.P. and Cristobal, S. (2005) *Peroxisomal proteomics, a new tool for risk assessment of peroxisome proliferating pollutants in the marine environment* Proteomics, **5**, 3954-2965

2j. Yeast

Nyathi, Y., De Marcos Lousa, C., van Roermund, C.W., Wanders, R.J.A., Johnson, B., Baldwin, S.A., Theodoulou, F.L. and Baker, A. (2010) *The Arabidopsis peroxisomal ABC transporter, Comatose, complements the Saccharomyces cerevisiae pxa1 pxa2Δ mutant for metabolism of long-chain fatty acids and exhibits fatty acyl-CoA-stimulated ATPase activity* J. Biol., Chem., **285**, 29892–29902

Nyathi, Y., Zhang, X., Baldwin, J.M., Bernhardt, K., Johnson, B., Baldwin, S.A., Theodoulou, F.L. and Baker, A. (2012) *Pseudo half-molecules of the ABC transporter, COMATOSE, bind Pex19 and target to peroxisomes independently but are both required for activity* FEBS Lett., **586**, 2280–2286

3. Self-generated gradient

3a. CHO cells

Honsho, M., Yagita, Y., Kinoshita, N. and Fujiki, Y. (2008) *Isolation and characterization of mutant animal cell line defective in alkyl-dihydroxyacetonephosphate synthase: Localization and transport of plasmalogens to post-Golgi compartments* Biochim. Biophys. Acta, **1783**, 1857-1865

Kobayashi, S., Tanaka, A. and Fujiki, Y. (2007) *Fis1, DLP1 and Pex11p coordinately regulate peroxisome morphogenesis* Exp. Cell Res., **313**, 1675-1686

Matsuzaki, T. and Fujiki, Y. (2008) *The peroxisomal membrane protein import receptor Pex3p is directly transported to peroxisomes by a novel Pex19p- and Pex16p-dependent pathway* J. Cell Biol. **183**, 1275–1286

3b. Hep-G2 cells

Morel, F., Rauch, C., Petit, E., Piton, A., Theret, N., Coles, B. and Guillouzo, A. (2004) *Gene and protein characterization of the human glutathione S-transferase kappa and evidence for a peroxisomal localization* J. Biol. Chem., **279**, 16246-16253

3c. Liver (rodent)

Costello, J.L., Castro, I.G., Camões, F., Schrader, T.A., McNeall, D., Yang, J., Giannopoulou, E-A., Gomes, S., Pogenberg, V. et al (2017) *Predicting the targeting of tail-anchored proteins to subcellular compartments in mammalian cells* J. Cell Sci., **130**, 1675-1687

He, D., Barnes, S. and Falany, C.N. (2003) *Rat liver bile acid CoA:amino acid N-acyltransferase: expression, characterization, and peroxisomal localization* J. Lipid Res., **44**, 2242-2249

Graham, J., Ford, T. and Rickwood, D (1994) *The preparation of subcellular organelles from mouse liver in self-generated gradients of iodixanol* Anal. Biochem., **220**, 367-373

Kurochkin, I.V., Mizuno, Y., Konagaya, A., Sakaki, Y., Schonbach, C. and Okazaki, Y. (2007) *Novel peroxisomal protease Tysnd1 processes PTS1- and PTS2-containing enzymes involved in β-oxidation of fatty acids* EMBO J., **26**, 835-845

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