

APPLICATION OF PIMONIDAZOLE – DETECTION

1. Formalin Fixed Paraffin Embedded Tissue.

Immunoperoxidase detection of pimonidazole binding is described in detail in Product Inserts for each Hypoxyprobe Kit.

2. Immunofluorescence on frozen sections

1. Bernsen HJ, Rijken PF, Peters JP, et al. Suramin treatment of human glioma xenografts; effects on tumor vasculature and oxygenation status. *J Neurooncol* 1999; 44: 129-36.
2. Bussink J, Kaanders JH, Rijken PF, Raleigh JA, Van der Kogel AJ. Changes in blood perfusion and hypoxia after irradiation of a human squamous cell carcinoma xenograft tumor line. *Radiat Res* 2000; 153: 398-404.
3. Bussink J, Kaanders JH, Strik AM, van der Kogel AJ. Effects of nicotinamide and carbogen on oxygenation in human tumor xenografts measured with luminescence based fiber-optic probes. *Radiother Oncol* 2000; 57: 21-30.
4. Bussink J, Kaanders JH, Strik AM, Vojnovic B, van Der Kogel AJ. Optical sensor-based oxygen tension measurements correspond with hypoxia marker binding in three human tumor xenograft lines. *Radiat Res* 2000; 154: 547-55.
5. Wijffels KI, Kaanders JH, Rijken PF, et al. Vascular architecture and hypoxic profiles in human head and neck squamous cell carcinomas. *Br J Cancer* 2000; 83: 674-83.
6. Lal A, Peters H, St Croix B, et al. Transcriptional response to hypoxia in human tumors. *J Natl Cancer Inst* 2001; 93: 1337-43.
7. Hoebbers FJ, Janssen HL, Olmos AV, et al. Phase 1 study to identify tumour hypoxia in patients with head and neck cancer using technetium-99m BRU 59-21. Vascular architecture, hypoxia, and proliferation in first-generation xenografts of human head-and-neck squamous cell carcinomas. Why do hypoxic cells behave badly? *Eur J Nucl Med Mol Imaging* 2002; 29: 1206-11.
8. Kaanders JH, Wijffels KI, Marres HA, et al. Pimonidazole binding and tumor vascularity predict for treatment outcome in head and neck cancer. *Cancer Res* 2002; 62: 7066-74.
9. Bussink J, Kaanders JH, van der Kogel AJ. Tumor hypoxia at the micro-regional level: clinical relevance and predictive value of exogenous and endogenous hypoxic cell markers. *Radiother Oncol* 2003; 67: 3-15.
10. Freeburg PB, Robert B, St John PL, Abrahamson DR. Podocyte expression of hypoxia-inducible factor (HIF)-1 and HIF-2 during glomerular development. *J Am Soc Nephrol* 2003; 14: 927-38.
11. Zoula S, Rijken PF, Peters JP, et al. Pimonidazole binding in C6 rat brain glioma: relation with lipid droplet detection. *Br J Cancer* 2003; 88: 1439-44.
12. Janssen HL, Hoebbers FJ, Sprong D, et al. Differentiation-associated staining with anti-pimonidazole antibodies in head and neck tumors. *Radiother Oncol* 2004; 70: 91-7.

13. van Laarhoven H, Lok J, Rijpkema M, et al. Relation between dynamic gadolinium uptake rate, tumor vasculature and tumor hypoxia in human colorectal liver metastases. *Proc Intl Soc Mag Reson Med*; 2004; 2004. p. 148.
14. Hoogsteen IJ, Peeters WJ, Marres HA, et al. Erythropoietin receptor is not a surrogate marker for tumor hypoxia and does not correlate with survival in head and neck squamous cell carcinomas. *Radiother Oncol* 2005; 76: 213-8.
15. Kempf VA, Lebedziejewski M, Alitalo K, et al. Activation of hypoxia-inducible factor-1 in bacillary angiomatosis: evidence for a role of hypoxia-inducible factor-1 in bacterial infections. *Circulation* 2005; 111: 1054-62.
16. Ljungkvist AS, Bussink J, Kaanders JH, et al. Hypoxic cell turnover in different solid tumor lines. *Int J Radiat Oncol Biol Phys* 2005; 62: 1157-68.
17. Troost EG, Bussink J, Kaanders JH, et al. Comparison of different methods of CAIX quantification in relation to hypoxia in three human head and neck tumor lines. *Radiother Oncol* 2005; 76: 194-9.
18. van Laarhoven HW, de Geus-Oei LF, Wiering B, et al. Gadopentetate dimeglumine and FDG uptake in liver metastases of colorectal carcinoma as determined with MR imaging and PET. *Radiology* 2005; 237: 181-8.
19. Jonathan RA, Wijffels KI, Peeters W, et al. The prognostic value of endogenous hypoxia-related markers for head and neck squamous cell carcinomas treated with ARCON. *Radiother Oncol* 2006; 79: 288-97.
20. Khan Z, Michalopoulos GK, Stolz DB. Peroxisomal localization of hypoxia-inducible factors and hypoxia-inducible factor regulatory hydroxylases in primary rat hepatocytes exposed to hypoxia-reoxygenation. *Am J Pathol* 2006; 169: 1251-69.
21. Ljungkvist AS, Bussink J, Kaanders JH, Wiedenmann NE, Vlasman R, van der Kogel AJ. Dynamics of hypoxia, proliferation and apoptosis after irradiation in a murine tumor model. *Radiat Res* 2006; 165: 326-36.
22. Troost EG, Laverman P, Kaanders JH, et al. Imaging hypoxia after oxygenation-modification: Comparing [(18)F]FMISO autoradiography with pimonidazole immunohistochemistry in human xenograft tumors. *Radiother Oncol* 2006.
23. van Laarhoven HW, Kaanders JH, Lok J, et al. Hypoxia in relation to vasculature and proliferation in liver metastases in patients with colorectal cancer. *Int J Radiat Oncol Biol Phys* 2006; 64: 473-82.
24. Bussink J, Kaanders JH, van der Kogel AJ. Microenvironmental transformations by VEGF- and EGF-receptor inhibition and potential implications for responsiveness to radiotherapy. *Radiother Oncol* 2007; 82: 10-7.
25. Degrossoli A, Bosetto MC, Lima CB, Giorgio S. Expression of hypoxia-inducible factor 1alpha in mononuclear phagocytes infected with *Leishmania amazonensis*. *Immunol Lett* 2007; 114: 119-25.
26. Donadieu E, Hamdi W, Deveze A, et al. Improved cryosections and specific immunohistochemical methods for detecting hypoxia in mouse and rat cochleae. *Acta Histochem* 2007; 109: 177-84.
27. Hoogsteen IJ, Marres HA, Bussink J, van der Kogel AJ, Kaanders JH. Tumor microenvironment in head and neck squamous cell carcinomas: predictive value and clinical relevance of hypoxic markers. A review. *Head Neck* 2007; 29: 591-604.

28. Hoogsteen IJ, Marres HA, van der Kogel AJ, Kaanders JH. The hypoxic tumour microenvironment, patient selection and hypoxia-modifying treatments. *Clin Oncol (R Coll Radiol)* 2007; 19: 385-96.
29. Kim YJ, Kang HH, Ahn JH, Chung JW. Hypoxic changes in the central nervous system of noise-exposed mice. *Acta Otolaryngol Suppl* 2007: 73-7.
30. Ljungkvist AS, Bussink J, Kaanders JH, van der Kogel AJ. Dynamics of tumor hypoxia measured with bioreductive hypoxic cell markers. *Radiat Res* 2007; 167: 127-45.
31. Wijffels KI, Marres HA, Peters JP, Rijken PF, van der Kogel AJ, Kaanders JH. Tumour cell proliferation under hypoxic conditions in human head and neck squamous cell carcinomas. *Oral Oncol* 2007; 44: 335-44.
32. Busk M, Horsman MR, Overgaard J. Resolution in PET hypoxia imaging: voxel size matters. *Acta Oncol* 2008; 47: 1201-10.
33. Bussink J, van der Kogel AJ, Kaanders JH. Patterns and levels of hypoxia in head and neck squamous cell carcinomas and their relationship to patient outcome: in regard to Evans et al. (*Int J Radiat Oncol Biol Phys* 2007;69:1024-1031). *Int J Radiat Oncol Biol Phys* 2008; 70: 1616.
34. Bussink J, van der Kogel AJ, Kaanders JH. Activation of the PI3-K/AKT pathway and implications for radioresistance mechanisms in head and neck cancer. *Lancet Oncol* 2008; 9: 288-96.
35. Gulliksrud K, Vestvik IK, Galappathi K, Mathiesen B, Rofstad EK. Detection of different hypoxic cell subpopulations in human melanoma xenografts by pimonidazole immunohistochemistry. *Radiat Res* 2008; 170: 638-50.
36. Riedl CC, Brader P, Zanzonico PB, et al. Imaging hypoxia in orthotopic rat liver tumors with iodine 124-labeled iodoazomycin galactopyranoside PET. *Radiology* 2008; 248: 561-70.
37. Troost EG, Laverman P, Philippens ME, et al. Correlation of [18F]FMISO autoradiography and pimonidazole immunohistochemistry in human head and neck carcinoma xenografts. *Eur J Nucl Med Mol Imaging* 2008; 35: 1803-11.
38. Wijffels KI, Marres HA, Peters JP, Rijken PF, van der Kogel AJ, Kaanders JH. Tumour cell proliferation under hypoxic conditions in human head and neck squamous cell carcinomas. *Oral Oncol* 2008; 44: 335-44.
39. Busk M, Horsman MR, Jakobsen S, et al. Can hypoxia-PET map hypoxic cell density heterogeneity accurately in an animal tumor model at a clinically obtainable image contrast? *Radiother Oncol* 2009; 92: 429-36.
40. Hoogsteen IJ, Lok J, Marres HA, et al. Hypoxia in larynx carcinomas assessed by pimonidazole binding and the value of CA-IX and vascularity as surrogate markers of hypoxia. *Eur J Cancer* 2009; 45: 2906-14.
41. Wijffels KI, Hoogsteen IJ, Lok J, et al. No detectable hypoxia in malignant salivary gland tumors: preliminary results. *Int J Radiat Oncol Biol Phys* 2009; 73: 1319-25.
42. Hoeben BA, Kaanders JH, Franssen GM, et al. PET of hypoxia with 89Zr-labeled cG250-F(ab')₂ in head and neck tumors. *J Nucl Med* 2010; 51: 1076-83.
43. Li XF, Sun X, Ma Y, et al. Detection of hypoxia in microscopic tumors using 131I-labeled iodo-azomycin galactopyranoside (131I-IAZGP) digital autoradiography. *Eur J Nucl Med Mol Imaging* 2010; 37: 339-48.

44. Masunaga S, Nagasawa H, Liu Y, et al. Evaluation of the radiosensitivity of the oxygenated tumor cell fractions in quiescent cell populations within solid tumors. *Radiat Res* 2010; 174: 459-66.
45. Santiago A, Eicheler W, Bussink J, et al. Effect of cetuximab and fractionated irradiation on tumour micro-environment. *Radiother Oncol* 2010; 97: 322-9.
46. Nijkamp MM, Hoogsteen IJ, Span PN, et al. Spatial relationship of phosphorylated epidermal growth factor receptor and activated AKT in head and neck squamous cell carcinoma. *Radiother Oncol* 2011; 101: 165-70.
47. Oehler C, O'Donoghue JA, Russell J, et al. ¹⁸F-fluoromisonidazole PET imaging as a biomarker for the response to 5,6-dimethylxanthenone-4-acetic acid in colorectal xenograft tumors. *J Nucl Med* 2011; 52: 437-44.
48. Rademakers SE, Lok J, van der Kogel AJ, Bussink J, Kaanders JH. Metabolic markers in relation to hypoxia; staining patterns and colocalization of pimonidazole, HIF-1 α , CAIX, LDH-5, GLUT-1, MCT1 and MCT4. *BMC Cancer* 2011; 11: 167.
49. Zaleska K, Bruechner K, Baumann M, Zips D, Yaromina A. Tumour-infiltrating CD11b⁺ myelomonocytes and response to fractionated irradiation of human squamous cell carcinoma (hSCC) xenografts. *Radiother Oncol* 2011.
50. Baker LC, Boulton JK, Walker-Samuel S, et al. The HIF-pathway inhibitor NSC-134754 induces metabolic changes and anti-tumour activity while maintaining vascular function. *Br J Cancer* 2012; 106: 1638-47.
51. Bayer C, Kielow A, Schilling D, et al. Monitoring PAI-1 and VEGF Levels in 6 Human Squamous Cell Carcinoma Xenografts During Fractionated Irradiation. *Int J Radiat Oncol Biol Phys* 2012; 84: e409-17.
52. Ding A, Kalaighanasundaram P, Ricardo SD, et al. Chronic treatment with tempol does not significantly ameliorate renal tissue hypoxia or disease progression in a rodent model of polycystic kidney disease. *Clin Exp Pharmacol Physiol* 2012.
53. Hoogsteen IJ, Marres HA, van den Hoogen FJ, et al. Expression of EGFR Under Tumor Hypoxia: Identification of a Subpopulation of Tumor Cells Responsible for Aggressiveness and Treatment Resistance. *Int J Radiat Oncol Biol Phys* 2012; 84: 807-14.
54. Janssens GO, Rademakers SE, Terhaard CH, et al. Accelerated radiotherapy with carbogen and nicotinamide for laryngeal cancer: results of a phase III randomized trial. *J Clin Oncol* 2012; 30: 1777-83.

3. Confocal Microscopy

1. Eikesdal HP, Bjerkvig R, Raleigh JA, Mella O, Dahl O. Tumor vasculature is targeted by the combination of combretastatin A-4 and hyperthermia. *Radiother Oncol* 2001; 61: 313-20.
2. Bassnett S, McNulty R. The effect of elevated intraocular oxygen on organelle degradation in the embryonic chicken lens. *J Exp Biol* 2003; 206: 4353-61.
3. Sminia P, Acker H, Eikesdal HP, et al. Oxygenation and response to irradiation of organotypic multicellular spheroids of human glioma. *Anticancer Res* 2003; 23: 1461-6.

4. Davies Cd, Lundstrom LM, Frengen J, et al. Radiation improves the distribution and uptake of liposomal doxorubicin (caelyx) in human osteosarcoma xenografts. *Cancer Res* 2004; 64: 547-53.
5. Khan Z, Michalopoulos GK, Stolz DB. Peroxisomal localization of hypoxia-inducible factors and hypoxia-inducible factor regulatory hydroxylases in primary rat hepatocytes exposed to hypoxia-reoxygenation. *Am J Pathol* 2006; 169: 1251-69.
6. Echevarria M, Munoz-Cabello AM, Sanchez-Silva R, Toledo-Aral JJ, Lopez-Barneo J. Development of cytosolic hypoxia and hypoxia-inducible factor stabilization are facilitated by aquaporin-1 expression. *J Biol Chem* 2007; 282: 30207-15.
7. Levesque JP, Winkler IG, Hendy J, et al. Hematopoietic Progenitor Cell Mobilization Results in Hypoxia with Increased Hypoxia-Inducible Transcription Factor-1{alpha} and Vascular Endothelial Growth Factor A in Bone Marrow. *Stem Cells* 2007; 25: 1954-65.
8. Thored P, Wood J, Arvidsson A, Cammenga J, Kokaia Z, Lindvall O. Long-Term Neuroblast Migration Along Blood Vessels in an Area With Transient Angiogenesis and Increased Vascularization After Stroke. *Stroke* 2007; 38: 3032-9.
9. Boutrid H, Pina Y, Cebulla CM, et al. Increased hypoxia following vessel targeting in a murine model of retinoblastoma. *Invest Ophthalmol Vis Sci* 2009; 50: 5537-43.
10. Chen J, Connor KM, Aderman CM, Willett KL, Aspegren OP, Smith LE. Suppression of retinal neovascularization by erythropoietin siRNA in a mouse model of proliferative retinopathy. *Invest Ophthalmol Vis Sci* 2009; 50: 1329-35.
11. Moon JW, Kim YJ, Khwarg SI, Chung H, Yu HG. Chorioretinal ischemia and angiogenic milieu following photodynamic therapy. *Curr Eye Res* 2010; 35: 314-21.
12. Ly A, Yee P, Vessey KA, Phipps JA, Jobling AI, Fletcher EL. Early inner retinal astrocyte dysfunction during diabetes and development of hypoxia, retinal stress, and neuronal functional loss. *Invest Ophthalmol Vis Sci* 2011; 52: 9316-26.

4. Flow Cytometry

1. Durand RE, Raleigh JA. Identification of nonproliferating but viable hypoxic tumor cells in vivo. *Cancer Res* 1998; 58: 3547-50.
2. Olive PL, Durand RE, Raleigh JA, Luo C, Aquino-Parsons C. Comparison between the comet assay and pimonidazole binding for measuring tumour hypoxia. *Br J Cancer* 2000; 83: 1525-31.
3. Eikesdal HP, Bjerkvig R, Raleigh JA, Mella O, Dahl O. Tumor vasculature is targeted by the combination of combretastatin A-4 and hyperthermia. *Radiother Oncol* 2001; 61: 313-20.
4. Vordermark D, Brown JM. Evaluation of hypoxia-inducible factor-1alpha (HIF-1alpha) as an intrinsic marker of tumor hypoxia in U87 MG human glioblastoma: in vitro and xenograft studies. *Int J Radiat Oncol Biol Phys* 2003; 56: 1184-93.
5. Bennewith KL, Durand RE. Quantifying transient hypoxia in human tumor xenografts by flow cytometry. *Cancer Res* 2004; 64: 6183-9.

6. Nelson DW, Cao H, Zhu Y, et al. A noninvasive approach for assessing tumor hypoxia in xenografts: developing a urinary marker for hypoxia. *Cancer Res* 2005; 65: 6151-8.
7. Vordermark D, Kraft P, Katzer A, Bolling T, Willner J, Flentje M. Glucose requirement for hypoxic accumulation of hypoxia-inducible factor-1alpha (HIF-1alpha). *Cancer Lett* 2005; 230: 122-33.
8. Durand RE, Aquino-Parsons C. The fate of hypoxic (pimonidazole-labelled) cells in human cervix tumours undergoing chemo-radiotherapy. *Radiother Oncol* 2006; 80: 138-42.
9. Jankovic B, Aquino-Parsons C, Raleigh JA, et al. Comparison between pimonidazole binding, oxygen electrode measurements, and expression of endogenous hypoxia markers in cancer of the uterine cervix. *Cytometry B Clin Cytom* 2006; 70: 45-55.
10. Koch CJ. Importance of antibody concentration in the assessment of cellular hypoxia by flow cytometry: EF5 and pimonidazole. *Radiat Res* 2008; 169: 677-88.
11. Brown AP, Chung EJ, Urick ME, et al. Evaluation of the fullerene compound DF-1 as a radiation protector. *Radiat Oncol* 2010; 5: 34.

5. Cytometry

1. Raleigh JA, Chou SC, Bono EL, Thrall DE, Varia MA. Semiquantitative immunohistochemical analysis for hypoxia in human tumors. *Int J Radiat Oncol Biol Phys* 2001; 49: 569-74.
2. Nordmark M, Loncaster J, Chou SC, et al. Invasive oxygen measurements and pimonidazole labeling in human cervix carcinoma. *Int J Radiat Oncol Biol Phys* 2001; 49: 581-6.
3. Nordmark M, Loncaster J, Aquino-Parsons C, et al. Measurements of hypoxia using pimonidazole and polarographic oxygen-sensitive electrodes in human cervix carcinomas. *Radiother Oncol* 2003; 67: 35-44.
4. Nordmark M, Loncaster J, Aquino-Parsons C, et al. The prognostic value of pimonidazole and tumour pO₂ in human cervix carcinomas after radiation therapy: A prospective international multi-center study. *Radiother Oncol* 2006; 80: 123-31.
5. Jankovic B, Aquino-Parsons C, Raleigh JA, et al. Comparison between pimonidazole binding, oxygen electrode measurements, and expression of endogenous hypoxia markers in cancer of the uterine cervix. *Cytometry B Clin Cytom* 2006; 70: 45-55.

6. Western Blot

1. Vordermark D, Brown JM. Evaluation of hypoxia-inducible factor-1alpha (HIF-1alpha) as an intrinsic marker of tumor hypoxia in U87 MG human glioblastoma: in vitro and xenograft studies. *Int J Radiat Oncol Biol Phys* 2003; 56: 1184-93.

2. Sato Y, Endo H, Okuyama H, et al. Cellular hypoxia of pancreatic beta-cells due to high levels of oxygen consumption for insulin secretion in vitro. *J Biol Chem* 2011; 286: 12524-32.

7. ELISA

1. Azuma C, Raleigh JA, Thrall DE. Longevity of pimonidazole adducts in spontaneous canine tumors as an estimate of hypoxic cell lifetime. *Radiat Res* 1997; 148: 35-42.

2. Arteel GE, Thurman RG, Raleigh JA. Reductive metabolism of the hypoxia marker pimonidazole is regulated by oxygen tension independent of the pyridine nucleotide redox state. *Eur J Biochem* 1998; 253: 743-50.