Step 7

Step 8

Step 9

Overview of Hypoxyprobe[™] Kits (NOTE: for REFERENCE ONLY - see Product Inserts and Product Data Sheets for detailed information)

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	Hypoxyprobe [™] Kit	Hypoxyprobe [™] Plus Kit	Hypoxyprobe TM Omni Kit	Hypoxyprobe [™] F6 Kit	Hypoxyprobe [™] Gemini Kit	Hypoxyprobe [™] Green Kit	Hypoxyprobe [™] Red 549 Kit	Hypoxyprobe [™] Red APC Kit	Hypoxyprobe [™] Biotin Kit	Hypoxyprobe [™] Red PE Kit	Hypoxyprobe [™] PAb27 Kit	Hypoxyprobe [™] Red 594 Kit	Hypoxyprobe [™] Cy 7 Kit	Hypoxyprobe [™] Pacific Blue Kit
Step 1	Pimonidazole introduced in-vitro or in-vivo	Pimonidazole introduced in-vitro or in-vivo	Pimonidazole introduced in-vitro or in-vivo	F6 introduced in-vitro or in- vivo	Pimonidazole and F6 introduced in-vitro or in-vivo	Pimonidazole introduced in-vitro or in-vivo	Pimonidazole introduced in-vitro or in-vivo	Pimonidazole introduced in-vitro or in-vivo	Pimonidazole introduced in-vitro or in-vivo	Pimonidazole introduced in-vitro or in-vivo	Pimonidazole introduced in-vitro or in-vivo	Pimonidazole introduced in-vitro or in-vivo	Pimonidazole introduced in-vitro or in-vivo	Pimonidazole introduced in-vitro or in-vivo
Step 2	Fix tissue sample	Fix tissue sample	Fix tissue sample	Option 1: observe hypoxia by MRI or NMR Option 2: Fix tissue sample,	Fix tissue sample	Fix tissue sample	Fix tissue sample	Fix tissue sample	Fix tissue sample	Fix tissue sample	Fix tissue sample	Fix tissue sample	Fix tissue sample	Fix tissue sample
Step 3	Add Mouse anti- pimonidazole (MAb) to tissue	Add Mouse FITC conjugated MAb antipimonidazole to tissue	Add rabbit anti- pimonidazole PAb to tissue	Add anti-F6 PAb to tissue Add anti-rabbit secondary antibody (Investigator's choice) to tissue	Add anti- pimonidazole PAb2627 to tissue	Add FITC linked mouse anti- pimonidazole MAb to tissue	Add Red 549-linked mouse anti- pimonidazole MAb to tissue	Add Red APC-linked mouse anti- pimonidazole MAb to tissue	Add biotin-linked mouse anti- pimonidazole MAb to tissue	Add Red PE-linked mouse anti- pimonidazole MAb to tissue	Add rabbit anti- pimonidazole PAb27 to tissue	Add Red 594-linked mouse anti- pimonidazole MAb to tissue	Add Cy 7-linked mouse anti- pimonidazole MAb to tissue	Add Pacific Blue- linked mouse anti- pimonidazole MAb to tissue
Step 4	Add secondary anti- mouse antibody to tissue	Add secondary anti- FITC HRP antibody to tissue	Add secondary anti- rabbit antibody (Investigator's choice) to tissue	observe hypoxic tissue	Add anti-rabbit secondary antibody (Investigator's choice) to tissue	observe hypoxic tissue	observe hypoxic tissue	observe hypoxic tissue	add streptavidin dye- linked secondary antibody (Investigator's choice) to tissue	observe hypoxic tissue	Add secondary anti- rabbit antibody (Investigator's choice) to tissue	observe hypoxic tissue	observe hypoxic tissue	observe hypoxic tissue
Step 5	observe hypoxic tissue	add chromogen to tissue	observe hypoxic tissue		Initial observation of hypoxic tissue				observe hypoxic tissue		observe hypoxic tissue			
Step 6		observe hypoxic tissue			Option 1: second observation of hypoxia via magnetic means (MRI/NMR) Option 2: Fix second									

Add PAbF6 primary antibody to tissue

Add anti-rabbit secondary antibody (Investigator's choice)

hypoxic tissue

Second observation of

to tissue

Chart Key				
MAb	mouse monoclonal antibody			
PAb	rabbit polyclonal antibody			
F6	abbreviation for the proprietary compound CCI-103F			
FITC	Fluoroscein Isothiocyanate; green fluorescing dye			
Red 549, Red 594,				
Red PE, Red APC	red fluorescing dyes			
Pacific Blue	blue fluorescing dye			
CY 7	near-infrared fluorescing dye			
Biotin	colorless protein that bonds with streptavidin-bound secondary			

Biotin colorless protein that bonds with streptavidin-bound secondary antibodies
Strepavidin colorless protein linked to dyes to facilitate bonding with biotin conjugated primary antibodies