











Opal™Multiplexed Immunofluorescence Protocol



αVasopressin (rabbit) αTyrosine Hydroxilase (rabbit) αCRH (rabbit)

- Zsuzsanna E. Tóth & Éva Mezey (2007). Simultaneous Visualization of Multiple Antigens with Tyramide Signal Amplification using Antibodies from the same Species. Journal of Histochemistry and Cytochemistry; 55 (6): 545-554
 E Stack et al (2014)
- E Stack et al (2014) Multipled Immunohistochemistry, imaging and quantitation: A review with as assessment of Tyramide Signal Amplification multispectral imaging and multiplex analysis Methods 70; 46-58

Heat-induced epitope retrieval (HIER) treatment (in this case using a microwave oven) between detection of each target eliminates antibody cross-reactivity
HEIR step removes 1° & 2° antibodies

PerkinElmer

- TSA signal stays because it is covalently bound
- > TSA: Tyramide Signal Amplification
- > Applicable to current IHC and IF methods
- Multispectral imaging
 - Enables quantitation of overlapping fluorophores
 - Removes limits to multiplexing
- Gives capacity to Tissue Analysis and quantitative stategies
- Scalable approach for higher level multiplexing





































































Opal and Cancer Immunology						PerkinElme
Quantitati	ve image anal	ysis - Results				
The relative distances of Lymphocytes from the tumor-stroma interface was analyzed						
Green ER+ cases		He	Blue Her2+ cases		Red triple negative cases	
				Average Cell Der	nsity (per sq mn	ו)
	Avg. CD8+ Dist. to Tumor Edge (µ)		CD4+		CD8+	
Case ID	Within Tumor	Within Stroma	Within Tumor	Within Stroma	Within Tumor	Within Stroma
BRER-01-6H	57.1	29.1	0	16	8	4
BRER-02-3G	8.2	32.6	0	24	10	140
BRER-03-2P	20.7	20.5	8	36	16	92
BRER-04-1N	18.2	34.2	0	20	8	56
BRHR-03-2C	N/A	18.8	0	0	0	8
BRHR-04-2J	14.4	40.2	0	144	63	688
and the second se					-	169
BRHR-09-2B	57.4	52.2	0	236	(400
BRHR-09-2B BRHR-10-2C	57.4 N/A	52.2 29.0	0	236 4	0	636
BRHR-09-2B BRHR-10-2C BRTN-21-2J	57.4 N/A 19.6	52.2 29.0 26.3	0 0 8	236 4 276	0 159	636 440
BRHR-09-2B BRHR-10-2C BRTN-21-2J BRTN-22-2L	57.4 N/A 19.6 17.8	52.2 29.0 26.3 24.3	0 0 8 0	236 4 276 4	0 159 47	636 440 1168
BRHR-09-2B BRHR-10-2C BRTN-21-2J BRTN-22-2L BRTN-23-3L	57.4 N/A 19.6 17.8 19.0	52.2 29.0 26.3 24.3 46.2	0 0 8 0 12	236 4 276 4 80	0 159 47 53	636 440 1168 424

































PerkinElmer Thank you for your attention! Multiplexing is a powerful tool to enable **PerkinElmer offers:** the simultaneous per-cell quantitation A selective and specific staining protocol for and distribution analysis of phenotypes multiplexed IHC within the tumor microenvironment of a Multispectral imaging systems for measuring single FFPE tissue section. individual labels Software to analyze cellular www.perkinelmer.com/cancer-immunology expression in discrete tissue compartments Technical expertise in reagents, imaging and analysis Contract discovery services for assay development and slide analysis Research Use Only lot for use in diagn

