WHITEPAPER

Classification and Cocktails: Melanoma Cocktails

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The skin is the largest human body organ. It is comprised of many different layers, each with its own unique function and susceptibility to abnormalities. Melanoma is a deadly skin disease known for its aggressive clinical behavior and high tendency to metastasize.[1] Most melanomas can be accurately diagnosed by examining histological features, including asymmetry, lack of circumscription, impaired maturation, hyper cellularity, cytological atypia, dermal mitoses, and pagetoid spread.[2] The distinction of benign abnormalities from melanoma is typically easy to establish, however, there is a subset of melanocytic tumors known for diagnostic challenges, development of late metastases, and difficulties in treatment.[1] These lesions include atypical spitzoid melanocytic proliferations, spindle cell melanomas mimicking atypical fibroxanthomas or other fibrohistiocytic lesions, nevoid melanomas, proliferative nodules versus melanoma in large congenital nevi, melanoma versus clear cell sarcoma, or other types of atypical nevus.[2] Spitzoid proliferations are one of the hardest to diagnose.[2] Research has led to the discovery of many immunohistochemistry (IHC) markers to help delineate melanoma diagnosis.

Biocare Medical offers a wide range of melanoma markers in single primary antibody and cocktailed antibody formats. Several of these melanoma cocktails include the combination of MART-1 (Melanoma Antigen Recognized by T cells 1) and tyrosinase, as MART-1 is specific for melanocytic lesions[3-4] and tyrosinase is involved in the regulation of melanogenesis in melanocytes.[1] In many melanomas, MART-1 is co-expressed with HMB45; however, studies have shown that MART-1 is more sensitive than HMB45 when labeling metastatic melanomas.[5] Additionally, tyrosinase has demonstrated to be a more sensitive marker when compared to HMB45 and MART-1 and also labels a higher percentage of desmoplastic melanomas than HMB45.[3] The combination of MART-1 and tyrosinase may also aid in identifying metastatic melanoma in sentinel lymph nodes.[6] SOX10 is another marker widely used in dermatopathology, as SOX10 has been expressed in the vast majority of desmoplastic and spindle cell melanomas and 100% of nevi.[7,8] Research suggest the combination of SOX10 with MART-1 and/or tyrosinase labels a higher percentage of melanomas in lymph nodes and in metastatic melanoma compared to S100.[9,10] Antibody cocktails containing PRAME may be used as an ancillary tool for melanoma margin assessment. PRAME is diffusely expressed in many primary and metastatic cutaneous melanomas, except for desmoplastic melanomas, and may aid in the distinction between nodal nevi from nodal metastatic melanoma.[11]

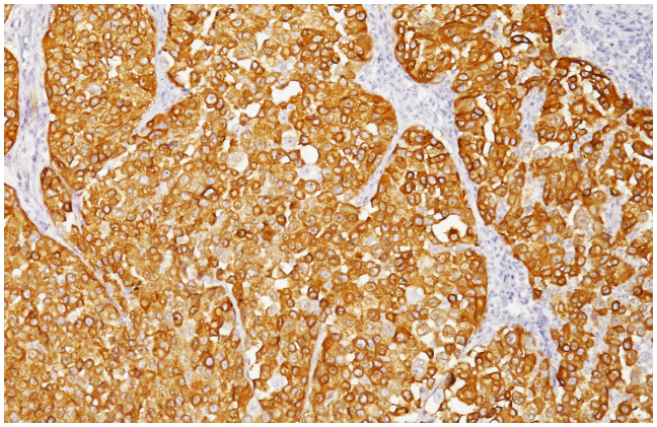
pHH3, Ki-67, and p16 are all cocktail components that may help assess melanocytic abnormalities.[1] Research shows Histone H3 phosphorylation at Serine10 (pHH3) can distinguish mitosis from apoptotic nuclei, which may correlate to a type of melanoma with worse prognosis.[1,12] Adding pHH3 to a cocktail containing MART-1 and Tyrosinase may allow identification of mitotic figures with improved specificity and time in the detection of mitotic figures in melanoma cells.[1] Ki-67 is a useful marker to index proliferation.[2] Researchers found Ki-67 is highly expressed in areas of non-spitzoid melanoma, while absent in Spitz nevi.[2] Combining KI-67 with Pan Melanoma (MART-1 + Tyrosinase) serves as a tool to identify the proliferation rate of melanocytic lesions in cases in which melanocytes are sparse, there are dense lymphocytic infiltrates, and melanocytes are admixed with fibroblasts. In general, a higher proliferative fraction is seen in melanoma than in melanocytic nevi. p16 INK4a is a tumor suppressor protein involved in the pathogenesis of a variety of malignancies. Studies have shown "loss of expression of the p16 in malignant melanoma was associated with tumor cell proliferation and invasive stage."[2] p16 has also proved to be a reliable marker for the differential diagnosis between lymph node nevi and melanoma metastasis, strongly reacting in lymph node nevi and lacking in melanoma deposits.[2]

The scope of melanoma malignancies might seem overwhelming. It is important to remember, however, research in IHC has provided a wide array of diagnostic markers that may aid in the interpretation of difficult melanocytic lesions. As concluded by Shidham et al., "The [Biocare] melanoma cocktail facilitated easy interpretation of melanoma micrometastases in sentinel lymph nodes with high interobserver agreement. There was improvement in detection rate with the cocktail as compared to MART-1 and Melan-A individually. Furthermore, this approach facilitates cost savings."[9] Research further suggests use of these biomarkers may aid in the identification and prognosis of melanoma diseases, speeding up the selection of beneficial target therapy.[1]

Looking to expand your melanoma marker menu? Biocare's melanoma cocktails are available as a standard predilutes in a variety of sizes and can be used both manually and on most automated IHC instrumentation. For more information, please call 1-800-799-9499 or visit our website: https://biocare.net/antibodies-organ/skin/.

HMB45 + Mart-1 + Tyrosinase Chart

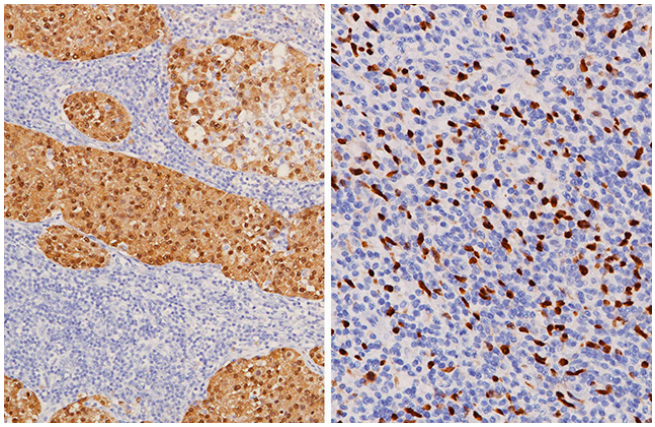
Antibody	anti-HMB45	anti-MART-1	anti-Tyrosinase
Clone	HMB45	M2-7C10 + M2-9E3	T311
Source	Mouse Monoclonal	Mouse Monoclonal	Mouse Monoclonal
Isotype	IgG1/kappa	IgG2b	IgG2a
Epitope/Antigen	HMB45	MART-1	Tyrosinase
Cellular Localization	Cytoplasmic	Cytoplasmic	Cytoplasmic



Melanoma labeled with HMB45 + MART-1 + Tyrosinase

Mart-1 + Tyrosinase + SOX10 Chart

Antibody	anti-MART-1	anti-Tyrosinase	anti-SOX10
Clone	M2-7C10 + M2-9E3	T311	BC34
Source	Mouse Monoclonal	Mouse Monoclonal	Mouse Monoclonal
Isotype	IgG2b	IgG2a	IgG1
Epitope/Antigen	MART-1	Tyrosinase	SOX10
Cellular Localization	Cytoplasmic	Cytoplasmic	Nuclear

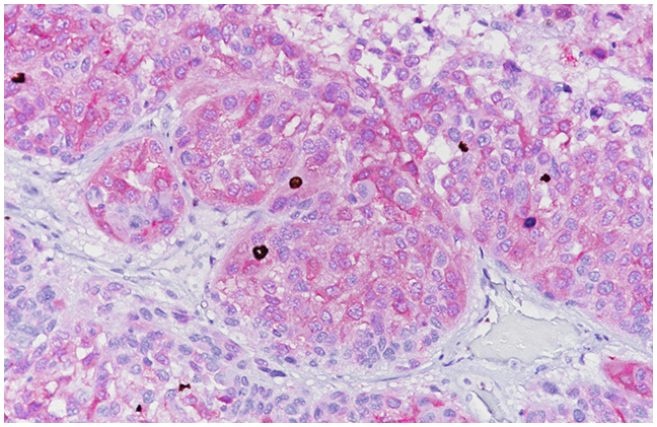


(Left) Metastatic melanoma stained with MART-1 + Tyrosinase (cytoplasmic, positive) and SOX10 (nuclear, positive)

(Right) Melanoma stained with MART-1 + Tyrosinase (cytoplasmic, negative) and SOX10 (nuclear, positive).

Mart-1 + Tyrosinase + pHH3 Chart

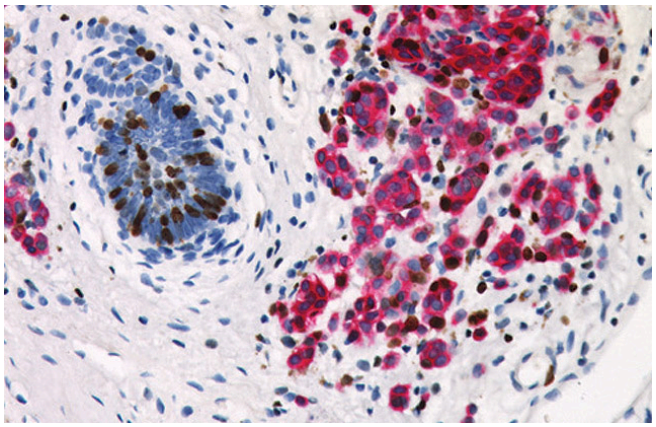
Antibody	anti-MART-1	anti-Tyrosinase	anti-pHH3
Clone	M2-7C10 + M2-9E3	T311	BC37
Source	Mouse Monoclonal	Mouse Monoclonal	Rabbit Monoclonal
Isotype	IgG2b	IgG2a	IgG
Epitope/Antigen	MART-1	Tyrosinase	PhosphoSer10 of Histone H3
Cellular Localization	Cytoplasmic	Cytoplasmic	Nuclear (mitotic figure)



Melanoma labeled with Biocare's melanoma cocktail (MART-1 + Tyrosinase + pHH3); pHH3 exhibiting [brown] mitotic figures.

Pan Melanoma + Ki-67 Chart

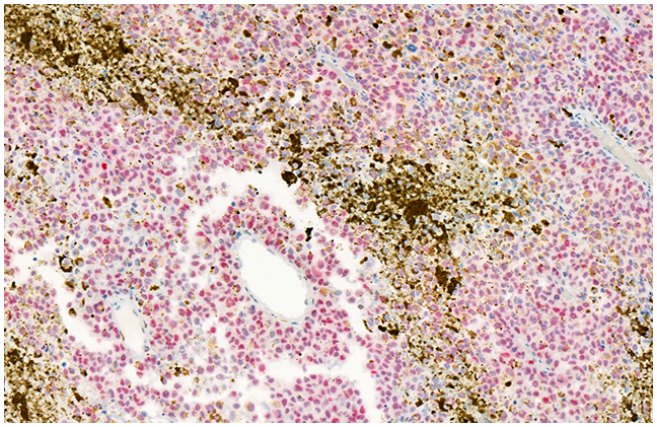
Antibody	anti-MART-1	anti-Tyrosinase	anti-Ki-67
Clone	M2-7C10 + M2-9E3	T311	SP6
Source	Mouse Monoclonal	Mouse Monoclonal	Rabbit Monoclonal
Isotype	IgG2b	IgG2a	IgG
Epitope/Antigen	MART-1	Tyrosinase	Ki-67
Cellular Localization	Cytoplasmic	Cytoplasmic	Nuclear



Melanoma labeled with Biocare's melanoma cocktail (Pan Melanoma + Ki-67); Ki-67 labeled with DAB [brown].

p16 + PRAME Chart

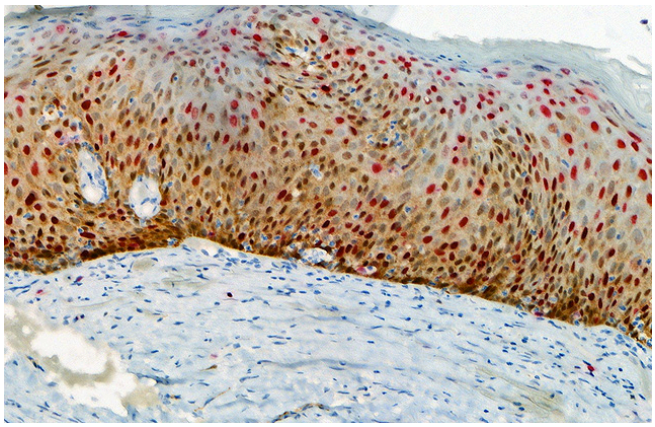
Antibody	anti-p16	anti-PRAME
Clone	BC42	EPR20330
Source	Mouse Monoclonal	Rabbit Monoclonal
Isotype	IgG1/kappa	IgG
Epitope/Antigen	p16 INK4a	PRAME
Cellular Localization	Nuclear and Cytoplasmic	Nuclear and Cell Membrane



Non-metastatic melanoma stained with PRAME (Red)

p16 + Ki-67 Chart

Antibody	anti-p16	anti-Ki-67
Clone	BC42	SP6
Source	Mouse Monoclonal	Rabbit Monoclonal
Isotype	IgG1/kappa	IgG
Epitope/Antigen	p16 INK4a	Ki-67
Cellular Localization	Nuclear and Cytoplasmic	Nuclear



Cervix labeled with Biocare's cocktail (p16 + Ki-67) p16 labeled with DAB [brown] and Ki-67 labeled with Fast Red [red]. When expressed in melanomas, research suggests, an increase in p16 labeling with a decrease in Ki-67 labeling indicates lesions may be nevus in nature.[2]

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