29 A Primer on Immunostains

The use of immunostains is a highly complex field in and of itself, and most residents will need to invest in a specialized text at some point. This chapter is meant as an introduction to the most commonly used stains so that you can at least follow the thread of conversation when the acronyms begin to fly. Stains are organized by the organ system in which they are most often used.

Blood Vessels (Endothelium)

Antibody	Normal tissues stained	When it is used
CD31	Endothelial cells and megakaryocytes (cytoplasmic and membranous), also macrophages	To identify endothelial differentiation or angiosarcoma; most specific endothelial marker
CD34	Endothelial cells, fibroblasts, and hematopoietic blasts (cytoplasmic and membranous)	To identify vascular sarcomas, Kaposi's sarcoma, GIST, solitary fibrous tumor, DFSP, epithelioid sarcoma, plus some other soft tissue tumors. Synovial sarcoma is negative
FVIII	Endothelial cells, megakaryocytes, platelets (cytoplasmic)	To identify endothelial differentiation, specific but not very sensitive

DFSP, dermatofibrosarcoma protuberans; GIST, gastrointestinal stromal tumor.

Brain and Meninges

Antibody	Normal tissues stained	When it is used
EMA	Epithelial, perineural, meningothelial cells (cytoplasmic or membranous)	To identify meningioma, perineuroma, chordoma, mesothelioma, sebaceous carcinoma, plus some sarcomas (synovial sarcoma, epithelioid sarcoma) and plasma cell neoplasms. Germ cell tumors (excluding some teratomas) are negative Entities that are EMA positive, keratin negative: meningioma, perineuroma, plasma cell myeloma

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Antibody	Normal tissues stained	When it is used
GFAP	Glial cells (cytoplasmic)	To identify astrocytoma, GBM, and ependymoma; also myoepithelial tumors of salivary gland. Oligodendroglioma and neuroblastoma are negative
NSE (neuronal-specific enolase)	Neuroectodermal and neuroendocrine cells (cytoplasmic)	To identify neural differentiation but not very specific (*not* the same as nonspecific esterase, an enzyme assay for heme path). Sensitive for neuroblastoma
S100	Glial cells, Schwann cells, dendritic and Langerhans cells, melanocytes, other mesenchymal cells (nuclear and cytoplasmic)	To identify cellular schwannoma, astrocytomas/GBM, granular cell tumor, chordoma, ependymoma, MPNST, and melanocytic lesions (all types). Breast cancer may also be positive
Synaptophysin	Neuroendocrine cells (cytoplasmic)	To identify carcinoids, paraganglioma, pheochromocytomas, small cell, medullary carcinoma of thyroid, neuroblastoma, islet cell tumors, others. Differentiates neural differentiation (positive from glial (negative)

GBM, glioblastoma multiforme; MPNST, malignant peripheral nerve sheath tumor.

Breast

Antibody	Normal tissues stained	When it is used
E-cadherin	Normal ductal and lobular cells (membranous)	Loss of staining identifies lobular carcinoma (in situ and invasive); ductal lesions are positive
ER and PR	Estrogen receptor (nuclear) and progesterone receptor (nuclear)	For breast cancer prognosis (predicts response to tamoxifen) and to identify metastatic breast cancer, some gynecologic tumors, and others
GCDFP	Apocrine metaplasia of breast and apocrine sweat glands (cytoplasmic)	To identify breast carcinoma, also sweat and salivary gland carcinoma
Her2Neu	Growth factor receptor that is only weakly expressed in normal epithelial cells (membranous and cytoplasmic)	To evaluate breast carcinomas (overexpression is a poor prognostic sign but can be treated with Herceptin)
Ki67	Any proliferating cell (nuclear)	To gauge mitotic activity for prognosis
Stains that identify myoepithelial cells		
α-Actin	Smooth muscle: myoepithelial cells, blood vessels, myofibroblasts (cytoplasmic)	To delineate myoepithelial layer and rule out invasive cancer
p63	Tumor suppressor gene (nuclear)	Stains myoepithelial cells but not endothelium and fibroblasts—cleaner stain than actin/SMMHC. Also stains metaplastic carcinoma
SMMHC (smooth muscle myosin-heavy chain)	Myoepithelial cells, blood vessels, myofibroblasts (cytoplasmic)	To delineate myoepithelial layer and rule out invasive cancer
CK903	Myoepithelial cells (cytoplasmic and membranous) and usual duct hyperplasia	To differentiate usual ductal hyperplasia (positive) from ductal carcinoma in situ (negative). Also stains metaplastic carcinoma.

Cytokeratins

Antibody	Normal tissues stained	When it is used
AE1-AE3	Wide panel of keratins stains most epithelial cells (cytoplasmic), except cytokeratins 8 and 18	To identify carcinomas in general; used in conjunction with Cam 5.2 to screen for carcinoma
Cam 5.2	Low- and intermediate-molecular weight keratins 8, 18, and 19 (cytoplasmic)	Used in conjunction with AE1/AE3 to screen for carcinoma. Also to identify hepatocellular carcinoma, some adrenal cortical tumors, and some carcinomas that are negative for other keratins (undifferentiated carcinoma)
CK5/6	Two specific high-molecular-weight keratins (cytoplasmic)	To differentiate squamous cell carcinoma (positive) or mesothelioma (positive) from adenocarcinoma (negative)
CK7	A specific low-molecular-weight cytokeratin (cytoplasmic)	CK7 and CK20 are used in combination to narrow the differential of carcinoma of unknown origin. CK7 is generally positive in above-the-diaphragm carcinomas (see next table on CK7 and CK20)
CK20	A specific low-molecular-weight cytokeratin (cytoplasmic)	Generally positive in below-the-diaphragm carcinomas and in Merkel cell carcinoma (see next table on CK7 and CK20)
CK903 (34ßE12)	High-molecular-weight keratin (cytoplasmic and membranous)	To identify prostatic basal cells (loss of staining indicates carcinoma), and transitional cell (urothelial) carcinoma (positive); also metaplastic breast carcinoma

	$CK20^+$	$CK20^-$
$CK7^+$	Urothelial carcinoma Pancreatic carcinoma Ovarian mucinous carcinoma	Breast carcinoma Lung carcinoma, non–small cell Ovarian serous carcinoma Endometrial carcinoma Epithelial mesothelioma Thymoma
$CK7^-$	Colorectal carcinoma Merkel cell carcinoma	Hepatocellular carcinoma Renal cell carcinoma, clear cell type Prostate carcinoma Neuroendocrine small cell carcinoma Squamous cell carcinoma

Germ Cell and Testis

Antibody	Normal tissues stained	When it is used
AFP	Fetal tissues (cytoplasmic)	To identify yolk sac tumor and HCC. May also stain other carcinomas
c-kit	Germ cells, mast cells, interstitial cells of Cajal (cytoplasmic or membranous)	To identify seminoma (membranous) and mature teratoma, plus GIST in stomach
EMA	Epithelial, perineural, meningothelial cells (membranous)	Should be negative in seminoma, yolk sac tumor, and embryonal carcinoma
β-hCG	Human chorionic gonadotropin β-chain (cytoplasmic) in syncytiotrophoblasts	To identify choriocarcinoma and germ cell tumors, some adenocarcinoma
HPL	Trophoblasts (cytoplasmic)	To identify germ cell tumors, moles, and choriocarcinoma, also some carcinomas
Ki-1 (CD30)	Activated lymphocytes	To identify embryonal carcinoma, Hodgkin's lymphoma, and ALCL
PLAP	Placenta (cytoplasmic)	To identify germ cell tumors, intratubular germ cell neoplasia, others; does not stain spermatocytic seminoma

ALCL, anaplastic large cell lymphoma; GIST, gastrointestinal stromal tumor; HCC, hepatocellular carcinoma.

Gynecologic

Antibody	Normal tissues stained	When it is used
CA-125		To identify nonmucinous ovarian carcinoma
β-hCG	Human chorionic gonadotropin β-chain (cytoplasmic) in syncytiotrophoblasts	To identify choriocarcinoma and germ cell tumors
HPL	Trophoblasts (cytoplasmic)	To identify germ cell tumors, moles, choriocarcinoma, and some carcinomas
Inhibin	Granulosa cells, Sertoli cells, others (cytoplasmic)	To identify sex cord stromal tumors (granulosa cell, Sertoli and Leydig) and moles, choriocarcinomas, fibrothecomas, and adrenal cortical tumors
Melcam (CD146)	Intermediate trophoblasts	To identify PSTT, choriocarcinoma
Mesothelin	Mesothelial cells (membranous)	To identify serous ovarian carcinoma, mesothelioma, and pancreatic carcinoma (also a target for immunotherapy)
p16	Cells infected by HPV (nuclear)	To identify HSIL and HPV lesions of cervix and to differentiate between endocervical (positive) and endometrial (negative) adenocarcinoma
p53	Tumor suppressor gene variant that should be absent in normal cells (nuclear)	To identify EIC and serous carcinoma of endometrium

EIC, endometrial intraepithelial carcinoma; HPV, human papillomavirus; HSIL, high-grade squamous intraepithelial lesion; PSTT, placental site trophoblastic tumor.

Heme Path

Antibody	Normal tissues stained	When it is used
ALK	Fusion protein expressed by only lymphomatous cells	Stains a subset of ALCL and DLBCL. Hodgkin's lymphoma is negative
bcl-2	Inhibits apoptosis, and normally turns *off* in a germinal center (membranous and cytoplasmic stain)	To differentiate follicular lymphoma (positive) from reactive follicles (negative). Also stains mantle cell lymphoma. Burkitt's lymphoma should be negative
bcl-6	Germinal center cells (cytoplasmic)	To identify lymphomas of follicular origin (FCC, Burkitt's lymphoma)
CD1a	Thymocytes (immature T cells) and Langerhans cells (membranous)	To identify Langerhans cell proliferations, T-LBL
CDs 3, 4, 5, 7, 8	T cells	To identify T-cell lymphomas and leukemias; CD4 is also dimly positive in monocytic/histiocytic lesions
CD10	Precursor B and T cells, granulocytes (membranous)	To identify FCC, ALL, LBL, Burkitt's lymphoma, and CML; MALTomas are negative
CD20	B cells (cytoplasmic and membranous)	Used as a pan-B-cell marker; stains B-cell lymphomas, but plasmacytomas are negative
CD15 (LeuM1)	Granulocytes and macrophages (membranous and dot-like perinuclear)	To identify RS cells (classic HD), some large T-cell lymphomas and mycosis fungoides
CD23	B cells, IgE receptor (membranous)	To identify SLL/CLL; mantle cell lymphomas are negative
CD30	Activated B and T cells, immunoblasts, other nonheme cell types (cytoplasmic, membranous)	To identify RS cells, ALCL, large B- and T-cell lymphomas
CD34	Hematolymphoid blasts, fibroblasts, and endothelial cells (cytoplasmic and membranous)	To identify blasts in the marrow in acute leukemias; also many soft tissue tumors
CD45 (CLA/LCA)	Lymphocytes, granulocytes, and histiocytes, but not plasma cells (cytoplasmic, membranous)	To identify poorly differentiated neoplasms as of hematopoietic origin
CD56	Natural killer cells (membranous)	To identify natural killer/T-cell lymphomas

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Antibody	Normal tissues stained	When it is used
CD68	Histiocytes/macrophages/monocytes, granulocytes, others (cytoplasmic, membranous)	To identify histiocytic origin; also stains soft tissue tumors
CD79a	B cells and plasma cells (membranous)	To identify B-cell neoplasms negative for other B-cell markers; stains B-ALL and B lymphomas, myelomas
CD138	Plasma cells (membranous), epithelial cells	To identify plasma cell neoplasms
Cyclin D1	Nuclear stain in mantle cell lymphoma	To identify mantle cell lymphoma
EBV EBER	EBV RNA in infected B cells (nuclear)	To identify EBV-related tumors, including nasopharyngeal carcinoma, posttransplantation/AIDS lymphomas; also mononucleosis
FVIII (vWF)	Stains megakaryocytes, platelets, and endothelial cells, (cytoplasmic)	To identify megakaryocytic leukemias
Hemoglobin	Hemoglobin in erythroid cells (cytoplasmic)	To identify erythroid leukemias (rare)
Kappa and lambda	Light chain of immunoglobulins in plasma cells and B cells (cytoplasmic)	Restricted kappa or lambda staining indicates a monoclonal population of B or plasma cells
Ki67	Any proliferating cell (nuclear)	To gauge mitotic activity and identify Burkitt's lymphoma (100% positivity)
MPO	Enzyme granules in myeloid-lineage cells (cytoplasmic)	To identify AML and myeloid sarcoma (chloroma)
TdT	Immature lymphocytes (nuclear)	To identify LBL and ALL

ALCL, anaplastic large cell lymphoma; ALL, acute lymphocytic leukemia; AML, acute myeloid leukemia; CML, chronic myeloid leukemia; DLBCL, diffuse large B-cell lymphoma; FCC, follicular center cell lymphoma; HD, Hodgkin's disease; LBL, lymphoblastic lymphoma; MALT, mucosa-associated lymphoid tissue; RS, Reed-Sternberg cells; SLL/CLL, small lymphocytic lymphoma/chronic lymphocytic leukemia.

Kidney and Bladder

Antibody	Normal tissues stained	When it is used
RCC (gp200/RTA)	Proximal renal tubules (cytoplasmic)	To identify renal cell carcinoma
TFE3	Transcription factor (nuclear)	To identify Xp11-translocation RCC and alveolar soft part sarcoma
TFE3B	Transcription factor (nuclear)	To identify t(6:11) renal cell carcinoma
Thrombomodulin	Both endothelial (cytoplasmic) and mesothelial (membranous) cells	To identify TCC, mesothelioma, some vascular tumors
WT-1	Tumor suppressor gene in developing nephrons; nephrogenic rests and adult glomerular podocytes (nuclear)	To identify Wilms' tumor; also mesothelioma, desmoplastic small round cell tumor

RCC, renal cell carcinoma; TCC, transitional cell (urothelial) carcinoma.

Liver, Pancreas, and Other Gastrointestinal

Antibody	Normal tissues stained	When it is used
α_1-antitrypsin	Histiocytes and reticulum cells (cytoplasmic)	To highlight globules of α_1-antitrypsin disease; not specific to a tumor
AFP	Fetal tissues (granular cytoplasmic)	To identify HCC and yolk sac tumors. May also stain other carcinomas

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Antibody	Normal tissues stained	When it is used
β-Catenin	APC-binding protein present in most cells (only nuclear staining is significant; indicates a mutation in APC or β-catenin)	To identify colon cancer, abdominal fibromatosis, and solid pseudopapillary tumor of pancreas (positive)
CD10	Liver canaliculi, brush border of small bowel, other tissues	A canalicular pattern in HCC
pCEA	Fetal tissues and adenocarcinomas (cytoplasmic)	A canalicular pattern in HCC (not seen with mCEA); also stains lung, colon, pancreatic carcinoma
DPC-4 clone B8	Most normal tissues (cytoplasmic)	To identify pancreatic carcinoma (55% of in situ or invasive cancers exhibit loss of staining)
EGFR	Hepatocytes, perineurium in peripheral nerves, squamous epithelium	Prediction of response to Erbitux (a monoclonal antibody) in advanced colon cancer
HepPar1 (OCH1E5)	Mitochondria in normal hepatocytes (granular cytoplasmic stain)	To identify HCC

Note: There are no immunostains that can differentiate well-differentiated HCC from normal liver tissue; the HCC stains are used to demonstrate hepatic differentiation in ambiguous or metastatic tumors. APC, activated protein C; HCC, hepatocellular carcinoma; mCEA, monocional carcinoembryonic antigen.

Lungs

Antibody	Normal tissues stained	When it is used
BerEP4	Epithelial cells (membranous)	To differentiate mesothelioma (negative) from carcinoma (positive)
mCEA and pCEA	Fetal tissues and mucin-secreting glandular tissues (cytoplasmic)	To differentiate mesothelioma (negative) from adenocarcinoma (positive)
Calretinin	Various neural and epithelial cells (cytoplasmic and nuclear)	To differentiate epithelial mesothelioma (positive) from carcinoma (negative)
LeuM1 (CD15)	Hematopoietic cells and some carcinomas (membranous and cytoplasmic)	To differentiate mesothelioma (negative) from adenocarcinoma (positive)
TTF-1	Transcription factor in lung and thyroid (nuclear)	To differentiate nonsquamous carcinoma of lung origin (including adenocarcinoma, small cell, and neuroendocrine/all positive) from nonpulmonary (negative)
WT-1	Mesothelium (nuclear)	To differentiate epithelial mesothelioma (positive) from carcinomas (negative)

Melanoma

Antibody	Normal tissues stained	When it is used
HMB45	Immature melanocytes (cytoplasmic)	To identify epithelioid melanoma, metastatic melanoma, angiomyolipoma, clear cell sarcoma, perivascular epithelioid cell tumors, and others
MART-1/ Melan-A (N2-7C10 clone)	Melanocytes (cytoplasmic)	To identify melanoma (mainly epithelioid), more sensitive than HMB45. Recognizes the same protein as Melan-A antibody
Melan-A (A103 clone)	Melanocytes (cytoplasmic)	To identify melanoma (mainly epithelioid), more sensitive than HMB45; also angiomyolipoma. Unlike MART-1, labels steroid cell tumors (adrenocortical tumors, Sertoli and Leydig cell tumors)
MitF	Melanocytes (nuclear)	To identify melanoma and melanocytic tumors, also angiomyolipoma
S100 protein	Melanocytes, glial cells, dendritic and Langerhans cells, other mesenchymal cells (nuclear and cytoplasmic)	To identify nevi and melanoma (all types, most sensitive), cellular schwannoma, granular cell tumor, glial neoplasms. Not used to screen lymph nodes, as normal dendritic cells are positive

Neuroendocrine and Endocrine

Antibody	Normal tissues stained	When it is used
Chromogranin	Neurosecretory granules (cytoplasmic, granular) in endocrine tissues and neurons	To differentiate pheochromocytoma (positive) from adrenal cortical carcinoma (negative) or to identify carcinoids, small cell, Merkel cell, and islet cell tumors
Inhibin	Adrenal cortical cells (cytoplasmic)	To identify adrenal cortical tumors, stromal sex cord tumors (granulosa cell, Sertoli and Leydig), fibrothecomas
Synaptophysin	Neuroendocrine cells (cytoplasmic), neuromuscular junction, Merkel cells	To identify carcinoids, paragangliomas, pheochromocytomas, small cell carcinoma, medullary carcinoma of thyroid, neuroblastoma, islet cell tumors, others
Various hormones	Cells that produce insulin, somatostatin, gastrin, glucagon, parathyroid hormone, etc. (cytoplasmic)	To identify products of neuroendocrine tumors, such as islet cell tumors and others

Prostate

Antibody	Normal tissues stained	When it is used
CK903 (34ßE12)	High-molecular-weight keratin (cytoplasmic and membranous) in basal cells	To identify prostatic basal cells (loss of staining indicates carcinoma) and TCC (positive)
p63	Prostatic gland basal cells (nuclear)	To identify basal cells (loss of staining indicates carcinoma)
PSA	Prostatic epithelium (cytoplasmic), but also salivary gland	To identify metastatic or ambiguous prostate cancer. Seminal vesicle is negative
PSAP (PAP)	Prostatic epithelium (cytoplasmic)	To identify metastatic or ambiguous prostate cancer and TCC; also stains rectal carcinoids
Racemase (p504s)	Prostatic carcinoma	To confirm prostate carcinoma; also stains nephrogenic adenoma of bladder

TCC, transitional cell (urothelial) carcinoma.

Soft Tissue

Antibody	Normal tissues stained	When it is used
α-Actin	Smooth muscle actin (cytoplasmic)	To identify smooth muscle differentiation, leiomyoma and leiomyosarcoma; rhabdomyosarcoma is usually negative
Actin (HHF-35)	Smooth, skeletal, and cardiac muscle, myoepithelial cells (cytoplasmic)	To identify muscle differentiation
c-kit	Mast cells, interstitial cells of Cajal (cytoplasmic and membranous)	To identify GIST, also seminoma, mature teratoma, and AML
CD34	Fibroblasts, endothelial cells, and hematopoietic blasts (cytoplasmic and membranous)	To identify GIST, SFT, DFSP, MPNST, and vascular sarcomas plus other soft tissue tumors
CD99 (O13)	A variety of mesenchymal cells (membranous)	To identify PNET/Ewing's sarcoma, lymphocytes in thymoma, plus other sarcomas and hematologic tumors. Neuroblastoma is negative
Desmin	Intermediate filaments in smooth, striated, and cardiac muscle (cytoplasmic)	To identify muscle differentiation, including rhabdomyosarcoma, some leiomyosarcomas, and others
EMA	Epithelial, perineural, meningeal cells (cytoplasmic or membranous)	To identify some sarcomas (synovial sarcoma, epithelioid sarcoma) plus chordoma, meningioma, mesothelioma, perineural tumors, and plasma cell tumors

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Antibody	Normal tissues stained	When it is used
FXIIIa and CD68	Fibrohistiocytic cells (cytoplasmic)	To identify fibrohistiocytic tumors, such as MFH and dermatofibroma (FXIIIa), giant cell tumor of tendon sheath
HMB45	Immature melanocytes (cytoplasmic)	To identify angiomyolipoma, clear cell sarcomas, PEComas, and others; also epithelioid and metastatic melanomas
Myogenin	Regenerating, but not normal, skeletal muscle (cytoplasmic)	To identify rhabdomyosarcoma
S100	Glial cells, melanocytes, dendritic and Langerhans cells, other mesenchymal cells (nuclear and cytoplasmic)	To identify cellular schwannoma, granular cell tumors, MPNST, chondrosarcoma, melanomas (all types), astrocytoma
Vimentin	Most mesenchymal cells (cytoplasmic), including fibroblasts, endothelium, smooth muscle	As an internal control for immunoreactivity and antigen preservation (the "pan-keratin" of soft tissue)
WT-1	Tumor suppressor gene in developing nephrons; nephrogenic rests and adult glomerular podocytes (nuclear)	To identify desmoplastic small round cell tumor, also Wilms' tumor

DFSP, dermatofibrosarcoma protuberans; GIST, gastrointestinal stromal tumor; MFH, malignant fibrous histiocytoma; MPNST, malignant peripheral nerve sheath tumor; PEComa, perivascular epithelioid cell tumor; PNET, primitive neuroectodermal tumor; SFT, solitary fibrous tumor.

Thyroid

Antibody	Normal tissues stained	When it is used
Calcitonin	C cells of the thyroid (cytoplasm and extracellular material)	To identify medullary carcinoma of thyroid
Thyroglobulin	Thyroid follicles (cytoplasmic)	To identify metastatic thyroid carcinoma
TTF-1	Transcription factor in lung and thyroid (nuclear)	To identify thyroid carcinoma, including follicular, papillary, and medullary; also nonsquamous carcinoma of lung (adenocarcinoma and small cell)

Differential Diagnoses

Spindle cell tumor: Differential diagnosis includes neural, muscle, fibrous, vascular, or other sarcoma, plus carcinoma and melanoma. Panel: S100, AE1/AE3, actins, desmin, CD34, c-kit.

Big pink cell tumor: Differential diagnosis includes melanoma, adrenal cortical carcinoma, renal cell carcinoma, hepatocellular carcinoma, thyroid Hurthle cell carcinoma, parathyroid, Leydig cell tumor.

Small round blue cell tumor: Differential diagnosis includes small cell carcinoma, Merkel cell, lymphoma, primitive neuroectodermal tumor/Ewing's sarcoma, alveolar rhabdomyosarcoma, desmoplastic small cell tumor, neuroblastoma, Wilms' tumor. Panel: CD45, CD99, desmin, NSE, CK20, chromogranin, myogenin, CD79a, TdT, WT-1, cytokeratin.

Alveolar pattern tumor: Differential diagnosis includes renal cell carcinoma, alveolar rhabdomyosarcoma, alveolar soft parts sarcoma, pheochromocytoma, granular cell tumor, melanoma.

Clear cell tumor: Differential diagnosis includes renal cell carcinoma, clear cell follicular thyroid carcinoma, clear cell lung carcinoma, clear cell hepatocellular carcinoma, adrenocortical carcinoma, malignant melanoma.