Polyclonal Rabbit Anti-Human CD117, c-kit

Code A4502

Intended use
For in vitro diagnostic use.

Polyclonal Rabbit Anti-Human CD117, c-kit is intended for use in immunohistochemistry (IHC). The antibody labels the transmembrane tyrosine kinase receptor CD117/c-kit, located in hematopoietic stem cells, melanocytes, mast cells, Cajal cells, germ cells, basal cells of skin, and mammary ductal epithelia.[1-5] The antibody is a useful aid for the classification of several cancers expressing c-kit, including mast cell diseases, acute myeloid leukemia (AML), small cell lung carcinoma (SCLC), and Ewing sarcoma, and gastrointestinal stromal tumors (GISTs).[1-4] Differential classification is aided by the results from a panel of antibodies. The clinical interpretation of any staining or its absence should be complemented by morphological studies using proper controls and should be evaluated within the context of the patient's clinical history and other diagnostic tests by a qualified pathologist. This antibody is intended to be used after the primary diagnosis of tumor has been made by conventional histopathology using nonimmunologic histochemical stains.

Synonyms
KIT; stem cell factor receptor; mast cell growth factor receptor; steel factor receptor; p145(c-kit)

Summary and explanation
The protooncogene c-*kit*, localized to human chromosome 4,[6] encodes a transmembrane receptor, CD117/c-kit, belonging to the class III receptor tyrosine kinase family, which includes the receptor for colony-stimulating factor 1, and the platelet-derived growth factor receptors type A and B. The extracellular region of these receptors consists of five immunoglobulin-like domains where the second and third loops are thought to be involved in ligand binding. The intracytoplasmic tyrosine kinase domain is split by a long hydrophilic insert between the ATP-binding region and the phosphotransferase active site. Receptor activation is accompanied by receptor dimerization, substrate phosphorylation and autophosphorylation, receptor internalization, activation of protein kinases and phospholipases, and transcription of different protooncogenes.[5] Generally, the c-kit tyrosine kinase receptor pathway has been shown to be important for tumor growth and progression in several cancers[4] and mutations in the c-*kit* gene leading to ligand-independent phosphorylation (activation) of the c-kit tyrosine kinase, and are believed to have a central pathogenetic role in e.g. gastrointestinal stromal tumors.[7]

Refer to *Dako General Instructions for Immunohistochemical Staining* or the detection system instructions of IHC procedures for: Principle of Procedure, Materials Required, Not Supplied, Storage, Specimen Preparation, Staining Procedure, Quality Control, Troubleshooting, Interpretation of Staining, General Limitations.

Reagent provided
Affinity-isolated rabbit antibody purified through antigen-bound, activated thiol AvidGel F affinity chromatography and provided in liquid form, in 0.05 mol/L Tris/HCl, 0.1 mol/L NaCl, 0.015 mol/L NaN_3, pH 7.2, 1% bovine serum albumin.

Protein concentration: See label on vial.

The protein concentration between lots may vary without influencing the optimal dilution. The titer of each individual lot is compared and adjusted to a reference lot to ensure a consistent immunohistochemical staining performance from lot-to-lot.

Immunogen
Peptide corresponding to amino acids 963 to 976 at the cytoplasmic C-terminal part of c-kit[1,5]

Specificity
In Western blotting of an extract of the small cell lung carcinoma cell line SY, that over-expresses c-kit mRNA, the antibody labels a band of 145 kDa corresponding to the c-kit protein. The labeled band is rather broad, from 120 to 155 kDa. An additional band of 100 kDa is also labeled.[1] In another study applying a different antibody to c-kit, a 100 kDa protein was, likewise, labeled. This labeling was abolished when the antibody was incubated with the synthetic c-kit peptide antigen used for immunization.[6]

In Western blotting, the antibody did not react with an extract of the adenocarcinoma cell line HS, that is without c-*kit* gene expression.[1]

Materials required, but not supplied
Refer to *Dako General Instructions for Immunohistochemical Staining* and/or the detection system instructions.

Precautions
1. For in vitro diagnostic use.
2. For professional users.
3. This product contains sodium azide (NaN_3), a chemical highly toxic in pure form. At product concentrations, though not classified as hazardous, sodium azide may react with lead and copper plumbing to form highly explosive build-ups of metal azides. Upon disposal, flush with large volumes of water to prevent metal azide build-up in plumbing.
4. As with any product derived from biological sources, proper handling procedures should be used.
5. Wear appropriate Personal Protective Equipment to avoid contact with eyes and skin.
6. Unused solution should be disposed of according to local, State and Federal regulations.

Storage
Store at 2–8 °C. Do not use after expiration date stamped on vial. If reagents are stored under any conditions other than those specified, the conditions must be verified by the user. There are no obvious signs to indicate instability of this product. Therefore, positive and negative controls should be run simultaneously with patient specimens. If unexpected staining is observed which cannot be explained by variations in laboratory procedures and a problem with the antibody is suspected, contact Dako Technical Support.

Specimen preparation
Paraffin sections: The antibody can be used for labeling paraffin-embedded tissue sections fixed in formalin. Pre-treatment of deparaffinized tissues with heat-induced epitope retrieval is recommended. For heat-induced epitope retrieval, the following solutions were found efficient: Target Retrieval Solution (Code S1700) and Target Retrieval Solution, pH 9 (Code S2368). The staining intensity was found to be reduced by a pretreatment using 0.01 mol/L citrate buffer, pH 6.0. Pre-treatment of tissues with Proteinase K destroyed the epitope. The tissue sections should not dry out during the treatment or during the following immunohistochemical staining procedure.

Staining procedure
These are guidelines only. Optimal conditions may vary depending on specimen type and preparation method, and should be validated individually by each laboratory. The performance of this antibody should be established by the user when utilized with other manual staining systems or automated platforms.

Dilution: Polyclonal Rabbit Anti-Human CD117, c-kit (Code A4502) may be used at a dilution range of 1:400 to 1:600 when applied on formalin-fixed, paraffin-embedded sections of gastrointestinal stromal tumor and using 20 minutes heat-induced epitope retrieval in Target Retrieval Solution (code S1700) and 30 minutes incubation at room temperature with the primary antibody. The recommended negative control is Rabbit Immunoglobulin Fraction, Solid-Phase Absorbed (Code X0936), diluted to a protein concentration identical to the concentration of the primary antibody.

Quality control: Positive and negative control tissues as well as negative control reagent should be run simultaneously using the same protocol as the patient specimens.

Visualization: EnVision+ HRP kits, e.g. Codes K4009, are recommended. Follow the procedure enclosed with the selected visualization kit.

Staining interpretation
The cellular staining pattern for anti-CD117, c-kit is membranous and/or cytoplasmic.

Performance characteristics
Normal tissues: The antibody labels breast epithelium, skin basal cells (seemingly melanocytes), spermatocytes, oocytes, tissue mast cells,[1] fibroblast-like bone lining cells[8] and intestinal Cajal cells.

Abnormal tissues: The antibody labeled 50/56 seminomas/dysgerminomas, 45/123 small cell carcinomas of the lung, 3/7 immature teratomas, 2/11 serous papillary ovarian adenocarcinomas, 2/14 malignant melanomas of skin, 2/18 neuroblastomas, 7/86 pulmonary squamous cell carcinomas, 1/18 basal cell carcinomas, 9/227 non-small cell cervical carcinomas, 1/35 pulmonary adenocarcinomas and 1/92 breast carcinomas.[1] The antibody was also tested on 365 soft tissue sarcomas. Tumors showing occasional immunoreactivity, in most cases focal labeling, were 3/5 melanotic schwannomas, 5/20 angiosarcomas, 4/20 metastatic melanomas, 4/20 Ewing sarcomas/malignant primitive peripheral neuroectodermal tumors, 2/10 perineuriomas, 2/20 extraskeletal myxoid chondrosarcomas. Rare tumor cells were labeled by the antibody in 1/10 low-grade fibromyxoid sarcomas and 1/20 desmoid fibromatosis. More than 35 consecutive GISTs were found to overexpress c-kit when examined with the antibody, results that were confirmed by immunoblotting. The labeling was typically diffuse and generally strong in these tumors.[3] The antibody also labeled activated bone marrow stem cells in bone marrow biopsies from cases of infiltrative malignant lymphomas and osteomyelitis. Labeling of activated bone marrow stem cells were more heterogeneous in cases of aplastic anaemia, myelodysplasia and metastasis from prostate or breast cancer.[8]

No labeling was observed in 17 large cell pulmonary carcinomas, 5 adenosquamous pulmonary carcinomas, 1 mucoepidermoid pulmonary carcinoma, 2 small cell type cervical carcinomas, 9 ovarian clear cell carcinomas, 4 ovarian mucinous cystadenocarcinomas, 4 endometrial carcinomas, 17 germ cell tumors excluding seminomas/dysgerminomas and immature teratomas, 355 gastrointestinal tract carcinomas, 59 liver carcinomas, 31 pancreas carcinomas, 7 gall bladder carcinomas, 16 bile duct carcinomas, 29 kidney carcinomas, 49 urinary bladder carcinomas, 159 prostate carcinomas, 14 adrenal carcinomas, 91 thyroid carcinomas, 45 skin carcinomas, 7 ganglioneuromas, 23 phaeochromocytomas, 12 paragangliomas, 7 thyroid medullary carcinomas, 1 retinoblastoma, 30 carcinoids, 10 pulmonary neuroendocrine tumors, 20 gastrointestinal tract neuroendocrine tumors, 3 Ewing tumors and 8 pancreatic islet cell tumors.[1] In soft tissue tumors, no labeling was observed in 40 leiomyosarcomas, 25 rhabdomyosarcomas, 20 myxofibrosarcomas, 10 myxoid/round cell liposarcomas, 10 dedifferentiated liposarcomas, 20 solitary fibrous tumors, 20 synovial sarcomas, 30 dermatofibrosarcoma protuberans, 20 schwannomas, 20 malignant peripheral nerve sheath tumors, 10 clear cell sarcomas, 10 low-grade endometrial stromal sarcomas and 5 follicular dendritic cell sarcomas.[3]

References

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Explanation of symbols

REF	Catalogue number		Temperature limitation	IVD	In vitro diagnostic medical device
	Manufacturer	LOT	Batch code		Contains sufficient for <n> tests
	Use by		Consult instructions for use	EC REP	Authorized representative in the European Community

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