Rabbit Anti-Human
Alpha-1-Fetoprotein
Code No. A 0008
Lot 020, Edition 09.07.01

Intended use
For in vitro diagnostic use.
This antibody is intended for laboratory use to identify qualitatively by light microscopy cells containing AFP in fetal and neoplastic tissues using immunocytochemical test methods. Positive results may aid in the classification of hepatocellular carcinomas, yolk sac tumours and mixed germ cell tumours. Differential identification is aided by the results from a panel of antibodies. Interpretation must be made within the context of the patient’s clinical history and other diagnostic tests by a qualified pathologist.

Presentation
DAKO Rabbit Anti-Human Alpha-1-Fetoprotein is the purified immunoglobulin fraction of rabbit antiserum.
Solvent: 0.1 mol/L NaCl, 15 mmol/L NaN₃.
Protein concentration: 2.2 g/L.
Antibody titre (SRI): 344 000 IU/mL (1).
DAKO Human Alpha-1-Fetoprotein Standard, code No. X 0900, lot 085 is used as reference for the titre determination.
The titre variation between different lots of A 0008 is less than 10%. This is achieved by adjusting the titre of each individual lot to match the titre of an antibody reference preparation kept at -80 °C.

Immunogen
Alpha-1-fetoprotein isolated from human cord serum.

Specificity
The antibody reacts with alpha-1-fetoprotein. Traces of contaminating antibodies have been removed by solid-phase absorption with human plasma proteins.
The specificity of the antibody has been ascertained as follows:
Crossed immunoelectrophoresis: Only the alpha-1-fetoprotein precipitation arch appears when using 12.5 µL DAKO A 0008 per square cm gel area against 2 µL of DAKO Alpha-1-Fetoprotein Standard, code No. X 0900, or 2 µL amniotic fluid. No precipitation arch is seen when the antibody is tested against 2 µL human plasma.
Staining: Coomassie Brilliant Blue.
ELISA: No significant reaction is seen in double antibody sandwich ELISA using DAKO Rabbit Anti-Human Alpha-1-Fetoprotein as catching antibody, normal human plasma as sample and DAKO Peroxidase-Conjugated Rabbit Anti-Human Alpha-1-Fetoprotein (code No. P 0128) for visualization.
Immunocytochemistry: When tested in the dilution of 1:100-1:300 by the PAP procedure on formalin-fixed, paraffin-embedded human tonsil, skin, fetal liver and hepatoma, a positive staining in hepatocytes of fetal liver and hepatoma is seen and no staining of other cells or tissue types has been observed.
Negative control: DAKO Rabbit Immunoglobulin Fraction, code No. X 0903, 1:1 000-1:3 000.

Application
The antibody is well-suited for gel immunoprecipitation techniques, RIA, ELISA (2) and immunocytochemistry (3).

GUIDELINE FOR DILUTION
Rocket immunoelectrophoresis: Antibody: 0.75 µL per square cm gel area. Standard: DAKO Alpha-1-Fetoprotein Standard, code No. X 0900*, 1+4 1+6 1+10 1+20. Dilution of samples (amniotic fluid): 1+1. Standard and sample volume: 5 µL.
RIA: DAKO Rabbit Anti-Human Alpha-1-Fetoprotein can be used in a dilution of 1:50 000.
ELISA: For double antibody sandwich ELISA of alpha-1-fetoprotein, DAKO "General ELISA Procedure" (Order No. 30 023) may serve as a guide. The procedure below is for the measurement of AFP in serum (range 5-200 µg/L).
Immunocytochemistry: 1:100-1:300 (PAP procedure).

* Human Alpha-1-Fetoprotein Standard, Code No. X 0900.
DAKO Alpha-1-Fetoprotein Standard is a human cord serum fraction. The content of alpha-1-fetoprotein is assessed against the WHO International standard preparation 72/225. As measured by rocket immunoelectrophoresis the concentration of alpha-1-fetoprotein is 90 890 international units per mL, standard error of measurement is 0.64%. When measured by single radial immunodiffusion the concentration is 85 960 international units per mL, standard error of measurement is 0.50%. One international unit is officially proposed to be 1.21 ng of alpha-1-fetoprotein.
Cross-reaction with other species

DAKO Rabbit Anti-Human Alpha-1-Fetoprotein cross-reacts with alpha-1-fetoprotein from sheep and swine as determined by immunocytochemistry.

Storage

2-8 °C.

Performance characteristics

The cellular staining pattern for anti-human AFP is cytoplasmic.

Normal tissues: Diffuse, cytoplasmic granular staining with anti-human AFP may be seen in cells of the embryonal yolk sac, fetal liver and the fetal intestinal tract. AFP is not present in normal non-fetal tissues.

Abnormal tissues: The AFP antigen may be found in hepatocellular carcinomas, yolk sac tumours, yolk sac elements in mixed germ cell tumours and some stomach carcinomas (4, 5). Seminomas, teratomas and pure choriocarcinomas do not express the AFP antigen (4). Since AFP is an oncofetal marker, the staining intensity will vary inversely with the degree of tumour differentiation.

References