

## Monoclonal Antibodies

# MAb 6D5 Against Proteins Overexpressed in Hepatocellular Carcinoma Cell Lines

### ANTIGEN USED FOR IMMUNIZATION

Apoptotic cells of human hepatocellular carcinoma (HCC) cell line HUH7 were used for immunization. Apoptosis was induced by ultraviolet-C (UVC) irradiation.

### METHOD OF IMMUNIZATION

UVC treated apoptotic HUH7 cells were injected into peritoneal cavity of 6- to 8-week-old female BALB/c mice at 3-week intervals. The mice received three booster injections. No adjuvant was used. Immunity was assessed by cell-ELISA. Fusion was performed 3 days after the last booster.

### PARENTAL CELL LINE USED FOR FUSION

Sp2/0 mouse myeloma cell line

### SELECTION AND CLONING PROCEDURE

Fused cells were plated into 96-well tissue culture plates in hybridoma growth medium consisting of high-glucose DMEM supplemented with 20% FCS, 100 IU penicillin, 100  $\mu$ g streptomycin, non-essential amino acids, and hybridoma selection reagents (hypoxanthine, aminopterin, thymidine [HAT]; Sigma, St. Louis, MO). Screening of hybrid cells was performed by cell-ELISA and clones with high absorbance values were subjected to subcloning by limited dilution.

### HEAVY AND LIGHT CHAINS OF IMMUNOGLOBULIN

IgG3,  $\kappa$  chain

### SPECIFICITY

We analyzed 15 HCC cell lines by Western blotting. Two ma-

ior proteins with molecular weights close to 80 kDa were found to be expressed either alone or concomitantly in all studied cells, except FLC4, which resembles normal hepatocytes.<sup>(1)</sup> A third protein band with the lowest molecular weight was observed only in Hep3B cells. The antibody is also suitable for the detection of its target proteins both in immunofluorescence and in immunohistochemistry. 6D5 MAb works well in frozen and paraffin-embedded human tissues.

### SPECIFIC ANTIGEN IDENTIFIED

Specific antigen not determined

### AVAILABILITY

Tissue culture supernatant	Yes ✓	No
Ascitic fluid	Yes ✓	No
Hybridoma cells	Yes	No ✓

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### REFERENCES

1. Arad U, Axelrod J, Ben-nun-Shaul O, Oppenheim A, and Galun E: Hepatitis B virus enhances transduction of human hepatocytes by SV40-based vectors. *J Hepatol* 2004; 40(3):520-526.