Human Ki-67 protein is strictly associated with cell proliferation. It is expressed during all active phases of the cell cycle (G1, S, G2, and mitosis) except resting cells (G0). The Ki-67 positive tumor cells (the Ki-67 labeling index) is often correlated with the clinical course of the disease. New monoclonal antibodies that react with the Ki-67 equivalent protein from rodents now extend the use of Ki-67 protein as a proliferation marker to laboratory animals in basic research. The most popular anti-Ki-67 clone is MIB1. However, MIB1 does not cross-react with mouse Ki-67. We generated a new ultra-specific mouse anti-Ki-67 monoclonal antibody (UMAB107) validated by 10K protein microarray chip. UMAB107 can be used to detect the expression of human and mouse Ki-67 by Western Blot (WB), Immunofluorescence (IF) and Immunohistostchemistry (IHC). From the IHC study on 627 human cancer tissues, UMAB107 shows high concordance of the staining patterns with MIB1.

Design & Methods

Figure 1. UltraMAB Development Procedure

- **Antibody generation:** Antibodies to Ki-67 were generated by immunizing Balb/c mice with a purified recombinant protein fragment corresponding to amino acids 1160-1493 of human MXK67 (NP_002408) produced in E.coli. Enriched B cells from immunized animals were fused to myeloma SP2/0 myeloma cells (ATCC) to generate hybridomas using standard techniques. Single clones were obtained by limiting dilution assays through 3-round screening (by ELISA and IHC) and subcloning, and then screened with different kinds of FFPE human tissues by IHC.

- **IHC:** IHC staining was performed on 4 μm thick paraffin tissue sections. Briefly, de-paraffinized slides were treated in 1 mM EDTA (pH 8.0 or pH9.0) antigen retrieval solution at 120 degree for 2.5 min in a high pressure cooker. Slides were incubated in primary antibodies for 90 min at room temperature (RT) without serum blocking. ZSBIO’s PV9000 detection system was used in IHC assays manually.

- **Protein array and assays:** Protein chips were printed and protein assays were executed as described (1).

Results

---- Validation Assays

**Screening Ki-67 specific antibodies by Immunohistochemical assay**

**Specificity analysis of UMAB107 by 10K protein microarray chip**

**Other applications of UMAB107**

**Results **** IHC comparison between UMAB107 and MIB1**

Table 1. Performance comparison between UMAB107 and MIB1 on FFPE human tissue samples

- UMAB107 can be used in multiple applications such as WB, IF, and IHC.
- UMAB107 can detect mouse Ki-67 with good performance and no obvious background staining. However, MIB1 does not recognize mouse Ki-67 and has abnormal staining patterns.
- UMAB107 showed high concordance of the staining patterns with MIB1.

Conclusions

1. UMAB107 can be used in multiple applications such as WB, IF, and IHC.
2. UMAB107 can detect mouse Ki-67 with good performance and no obvious background staining. However, MIB1 does not recognize mouse Ki-67 and has abnormal staining patterns.
3. UMAB107 showed high concordance of the staining patterns with MIB1.

References