Selecting Antibodies To Use on Milo

Introduction

Like all antibody-based detection methods, Single-Cell Westerns™ with Milo™ require antibody selection and assay optimization when detecting new targets and analyzing new sample types. This tech note provides guidelines on qualities that make an antibody more likely to work on Milo to help you select antibodies from a commercial catalog. We also highlight four robust and well-validated internal control antibodies from different host species and molecular weight ranges so you can easily combine antibodies against your targets of interest with an internal control. Use this information to design your next Single-Cell Western assay with ease.

Selecting An Internal Control Antibody

It's always important to probe your cells for an internal standard like a housekeeping protein. The internal standard peak confirms that a cell was captured in a particular well, serves as a molecular weight reference, and (for some biological questions) can be used to normalize target signal. We strongly recommend using one of the antibodies listed in **Table 1** to probe for an internal standard in all Single-Cell Western assays as these antibodies have been validated in numerous diverse sample types.

TARGET	HOST	SUPPLIER, CATALOG #	EXPECTED MOLECULAR WEIGHT
β–tubulin	Mouse	GenScript A-01717-40	51 kDa
β–tubulin	Rabbit	Abcam, ab6046	51 kDa
GAPDH	Goat	Sigma, SAB2500450	74 kDa*
Histone H3	Rabbit	Cell Signaling, 4499	17 kDa

FIGURE 1. List of validated antibodies that can be used as a Single-Cell Western assay internal control. (*) indicates target typically observed as a dimer on Milo.

Selecting Antibodies Against Protein Targets of Interest

When selecting an antibody to detect a new target protein, look at the specific applications that a particular antibody has been validated for on the manufacturer's website. If multiple antibodies from reputable vendors are available, we recommend selecting an antibody that has been validated for both denatured and native conformational assays. In internal tests, commercial antibodies that are validated for western blotting have been successful on Milo approximately ⅔ of the time. Antibodies validated for the combination of western blotting, flow cytometry and immunocytometry/ immunofluorescence have been successful on Milo >⅘ of the time.

To see if an antibody has already been validated on Milo against your target of interest, please visit www.proteinsimple.com/milo.

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