Streamlined Mouse Tumor Processing with STEMprep™: Automated, Efficient, and Reliable

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INTRODUCTION

The tumor microenvironment is highly heterogeneous, composed of diverse cell types with distinct phenotypes and characteristics. Gaining insights into these differences is essential for advancing cancer immunotherapy and research. Reliable results demand high-quality samples and standardized tissue processing, however, generating single-cell suspensions from solid tumors is challenging due to variations in tumor type, size, firmness, and developmental stage. Balancing enzymatic and mechanical dissociation is critical to preserve cell viability and yield. We developed the STEMprep™ Mouse Tumor Dissociation Kit for use with the STEMprep™ Tissue Dissociator, which features a user-friendly interface and specially designed sample tubes. This system includes an optimized enzyme formulation and a single protocol that works across diverse tumor types. Our findings demonstrate consistently high cell viability and yield in tumors with varying firmness: hard 4T1 mammary carcinoma, medium CT26 colon carcinoma, and soft B16 melanoma. This method maintains cell integrity and epitope availability for downstream analysis. Furthermore, STEMprep™-processed samples are compatible with EasySep™ magnetic cell isolation technology, and are functional in T cell suppression assays, enhancing tumor research workflows with efficiency, consistency, and reliability.

METHODS

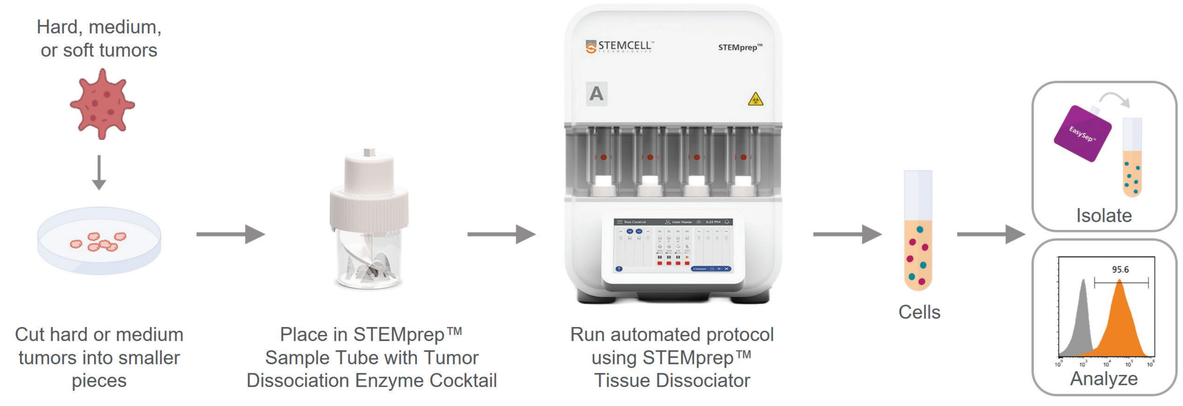


FIGURE 1. Automated Tumor Dissociation Workflow Using the STEMprep™ System

The STEMprep™ System can efficiently generate single-cell suspensions from various tumor types, including hard, medium, and soft tumors. STEMprep™-processed cells can be used in various downstream applications, including cell separation, flow cytometry, and cell culture.

RESULTS

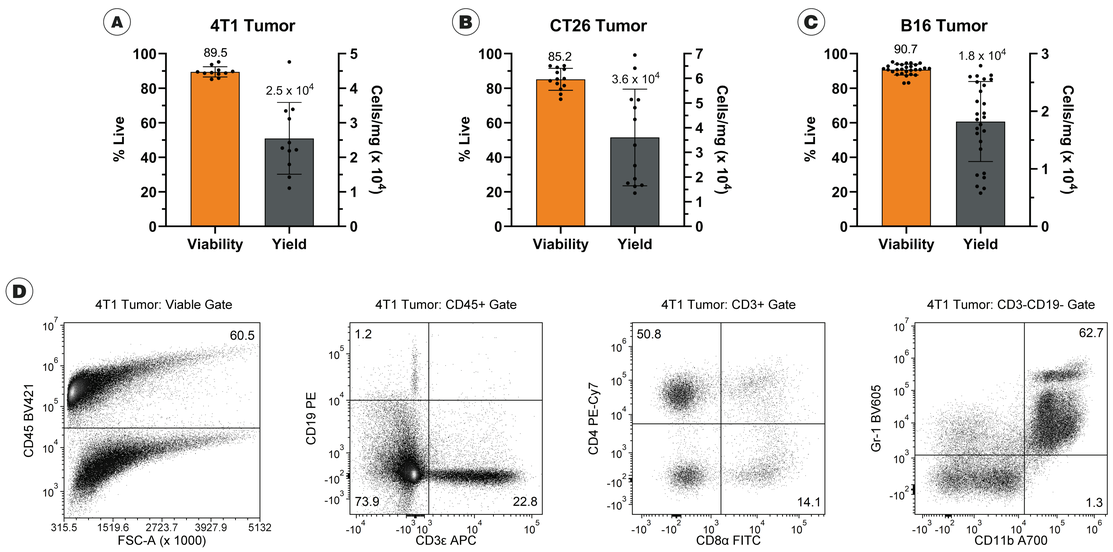


FIGURE 2. The STEMprep™ System Enables Reliable Automated Mouse Tumor Dissociation

Primary solid tumors were generated by subcutaneous injection of tumor cell lines into the flanks of mice. Mouse tumor tissues were processed into single-cell suspensions using the STEMprep™ Mouse Tumor Dissociation Kit. Cells dissociated from **(A)** hard 4T1 mammary tumors (n = 11), **(B)** medium CT26 colon carcinoma tumors (n = 13), and **(C)** soft B16 melanoma tumors (n = 28) were analyzed by flow cytometry to assess cell viability and yield. **(D)** Immune cell subsets in 4T1 tumors were assessed by flow cytometry. Data are presented as mean ± SD.

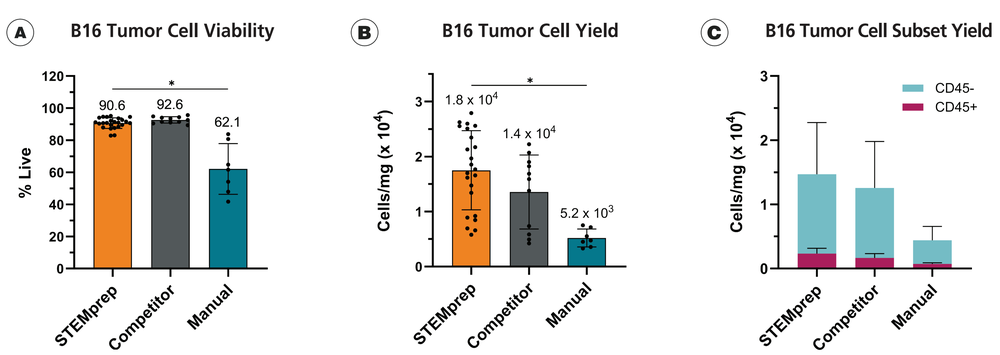


FIGURE 3. The STEMprep™ Mouse Tumor Dissociation Kit Achieves High Cell Viability and Yield

Mouse B16 tumor tissue was processed using the STEMprep™ Mouse Tumor Dissociation Kit, a competitor's automated system, or a standard manual dissociation method. **(A)** Viability of nucleated cells. **(B)** Yield of viable cells per mg tissue. **(C)** Yield of CD45+ immune and CD45- non-immune cell subsets. Data are presented as mean ± SD (n = 7 - 24); * $p < 0.05$, one-way ANOVA with Tukey's multiple-comparisons test.

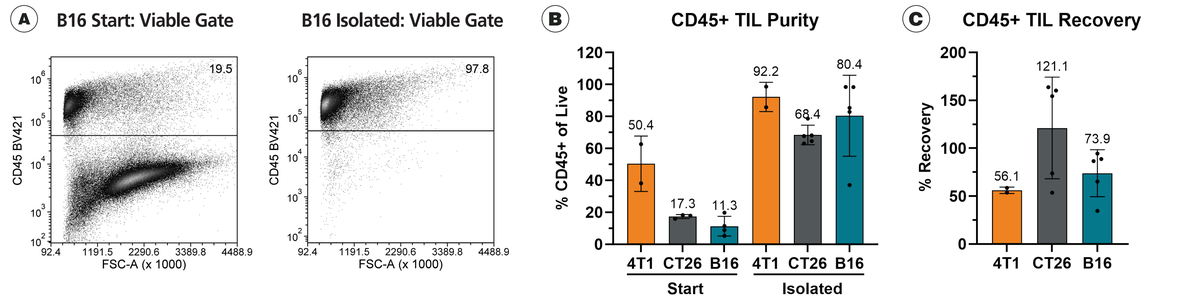
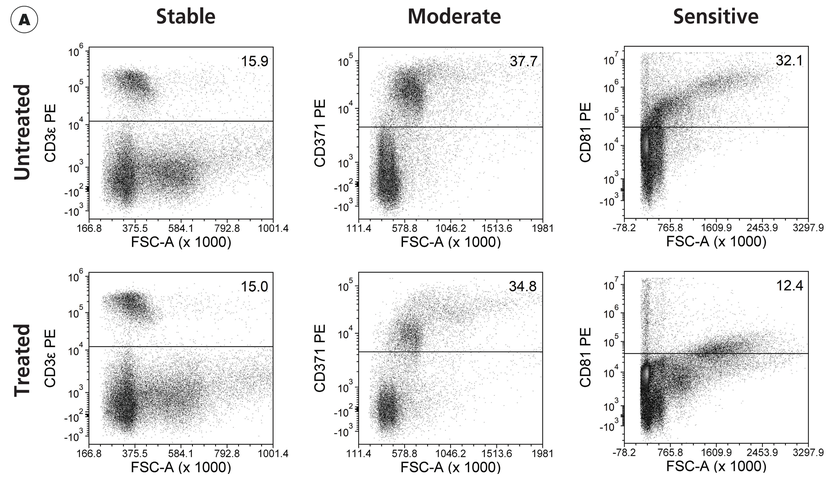


FIGURE 4. Isolation of CD45+ Tumor-Infiltrating Leukocytes from STEMprep™-Processed Mouse Tumors

Mouse 4T1, CT26, and B16 tumors were processed into single-cell suspensions using the STEMprep™ Mouse Tumor Dissociation Kit. Tumor-infiltrating leukocytes (TILs) were subsequently enriched using the EasySep™ Mouse TIL (CD45) Positive Selection Kit. **(A)** Representative flow cytometry plots of CD45+ TILs before and after isolation from B16 tumors. **(B)** Purity and **(C)** recovery of CD45+ TILs. Data are presented as mean ± SD (n = 2 - 5).



Category	% Epitopes	Conclusion
Stable	87.9	Detectable
Moderate		
Sensitive	12.1	Impacted (requires optimization or use of other clones)

FIGURE 5. Major Cell Surface Epitopes are Preserved After Incubation with the STEMprep™ Tumor Dissociation Enzyme Cocktail

Surface markers of immune and tumor/non-immune cells were analyzed using a mixture of mouse spleen, lung, and bone marrow cells, along with mouse tumor cell lines (4T1, CT26, and B16). One part was left untreated and labeled with CellTrace™ Violet dye (Untreated), while the other part was treated with STEMprep™ Mouse Tumor Dissociation cocktail (Treated) and incubated at 37°C for 40 minutes. The two parts were recombined at a 1:1 ratio. Cells were analyzed for ~200 surface antigens using BioLegend LEGENDScreen™ Mouse PE Kit. **(A)** Representative flow plots showing the sensitivity of select epitopes to the tumor enzyme cocktail; results were categorized as stable (CD3, clone 145-2C11), moderately affected (CD371, clone 5D3), and sensitive (CD81, clone Eat-2). **(B)** Summary of marker sensitivity.

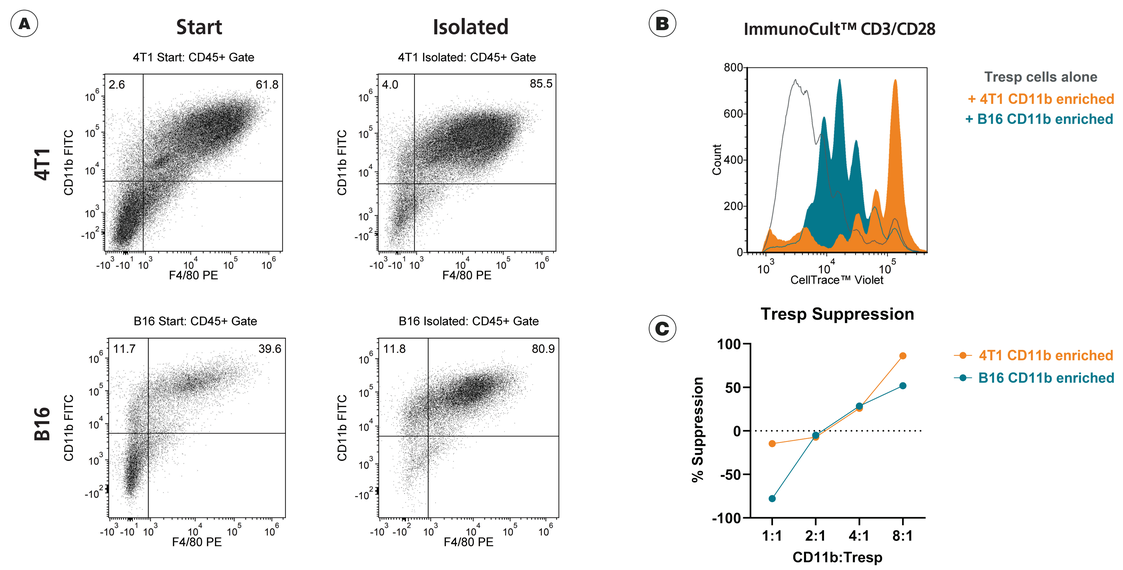


FIGURE 6. Intratumoral CD11b+ Cells Retain Immunosuppressive Function Downstream of STEMprep™ and EasySep™ Enrichment

Mouse 4T1 and B16 tumors were dissociated using the STEMprep™ Mouse Tumor Dissociation Kit followed by enrichment of CD11b+ cells. Splenic CD3+ T responder (Tresp) cells were labeled with CellTrace™ Violet, activated with the ImmunoCult™ Mouse T Cell CD3/CD28 soluble activator, and cultured either alone or with CD11b-enriched cells at increasing CD11b:Tresp cell ratios at 37°C for 4 days. **(A)** Purity of CD11b+ cells before and after enrichment with the EasySep™ Mouse CD11b Positive Selection Kit II. **(B)** Representative histograms of Tresp proliferation in the presence or absence of CD11b enriched cells. **(C)** Summary of Tresp suppression by CD11b enriched cells from 4T1 and B16 tumors. Data are presented as the mean of 2 independent experiments.

Summary

- The STEMprep™ Mouse Tumor Dissociation Kit is compatible with various tumor types (4T1, CT26, and B16).
- Automated dissociation with the STEMprep™ System enhances tumor cell viability and yield compared to manual processing.
- STEMprep™-processed tumor cells maintain cell surface antigen integrity and suitability for subsequent cell separation and T-cell co-culture workflows.