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No-Wash No-Lyse Techniques:

Simplifying Flow Cytometry

Conventional flow cytometry has relatively limited sensitivity, meaning that heterogeneous cell samples often have to be purified through lysis and wash steps prior to analysis. Technological advancements have enabled "no-wash, no-lyse" workflows for more precise and faster flow cytometry analysis.

No-Wash/No-Lyse Techniques

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No-Wash/No-Lyse Techniques: Simplifying Flow Cytometry

Conventional flow cytometry has relatively limited sensitivity, meaning that heterogeneous cell samples often have to be purified through lysis and wash steps prior to analysis. Technological advancements have enabled no-wash/no-lyse workflows for more precise and faster flow cytometry analysis.

What are No-Wash/No-Lyse Techniques?

Wash and lysis steps remove unwanted cells from heterogeneous cell samples, leaving purified populations for analysis. However, these steps can cause significant collateral damage to investigated cells [1-3], which contributes to interexperimental and interlaboratory variation [4].

Improved technology has resulted in no-wash/no-lyse protocols for detecting cells without labor-intensive and potentially deleterious wash- or lysis-mediated purification. No-wash/no-lyse workflows have been used to generate accurate, reproducible results for a variety of applications [2,4-6].

No-Wash/No-Lyse Applications

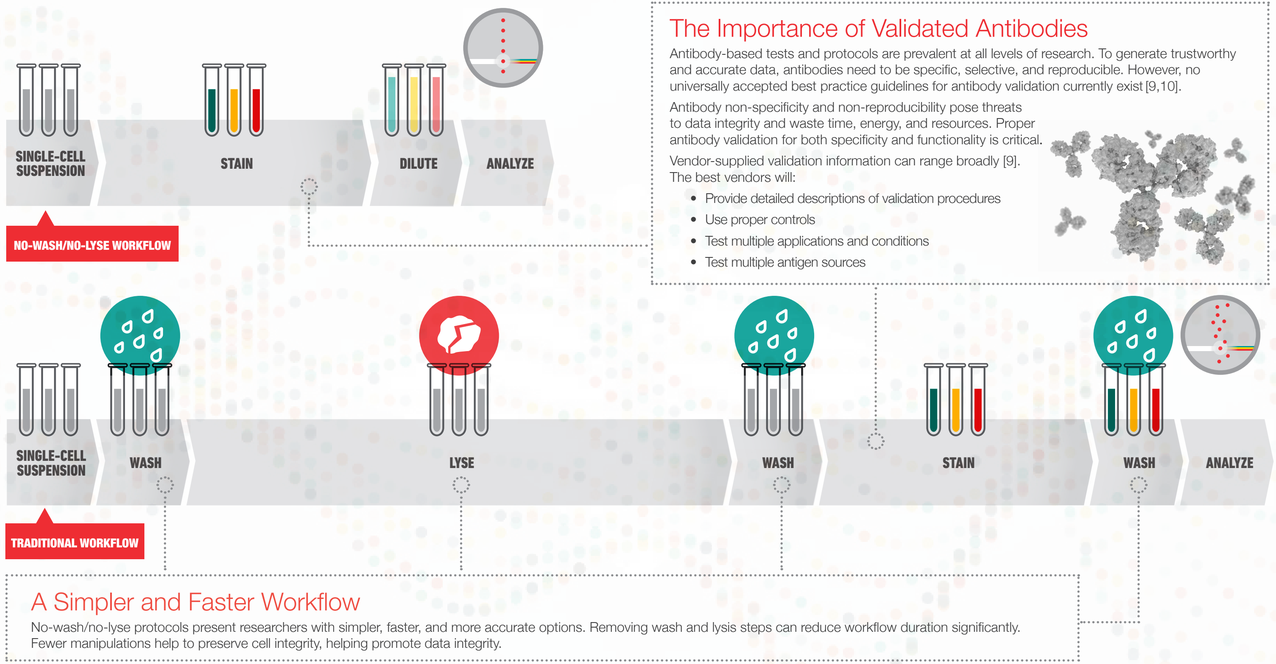
No-wash/no-lyse protocols have become popular for a range of applications in a variety of fields.

Used for:

- **Determining absolute cell counts**
- **Human blood diagnostic analysis [4,6]**
- **Immunophenotyping [11]**
- **Identifying changes in protein expression/phosphorylation [2] or enzyme activity/interactions [5]**

Used in:

- **Vaccine development**
- **Longitudinal studies**
- **Oncology**
- **Cardiovascular disease**



A Simpler and Faster Workflow

No-wash/no-lyse protocols present researchers with simpler, faster, and more accurate options. Removing wash and lysis steps can reduce workflow duration significantly. Fewer manipulations help to preserve cell integrity, helping promote data integrity.

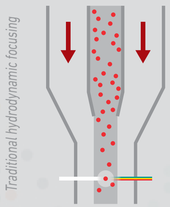
The Importance of Validated Antibodies

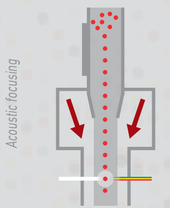
Antibody-based tests and protocols are prevalent at all levels of research. To generate trustworthy and accurate data, antibodies need to be specific, selective, and reproducible. However, no universally accepted best practice guidelines for antibody validation currently exist [9,10].

Antibody non-specificity and non-reproducibility pose threats to data integrity and waste time, energy, and resources. Proper antibody validation for both specificity and functionality is critical.

Vendor-supplied validation information can range broadly [9]. The best vendors will:

- Provide detailed descriptions of validation procedures
- Use proper controls
- Test multiple applications and conditions
- Test multiple antigen sources





Technology Marches On

No-wash/No-lyse workflows take advantage of new flow cytometry technologies and techniques.

Acoustic Cytometry

Acoustic radiation pressure can focus particles into a single tight line for flow cytometric optical interrogation. This acoustic focusing eliminates the need for sample dilution and provides superior flow control compared to conventional hydrodynamic focusing, resulting in greater sensitivity, precision, throughput, and applicative range [7,8].

True Volumetric Absolute Counting (TVAC)

TVAC is based on the principle that c = N / V, where the particle concentration (c) is equal to the counted number (N) divided by the volume (V). TVAC directly measures V, yielding an absolute value and providing better precision than conventional volume assessment using calibration counting beads [1].

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