

# Flow Cytometry Validated Antibodies

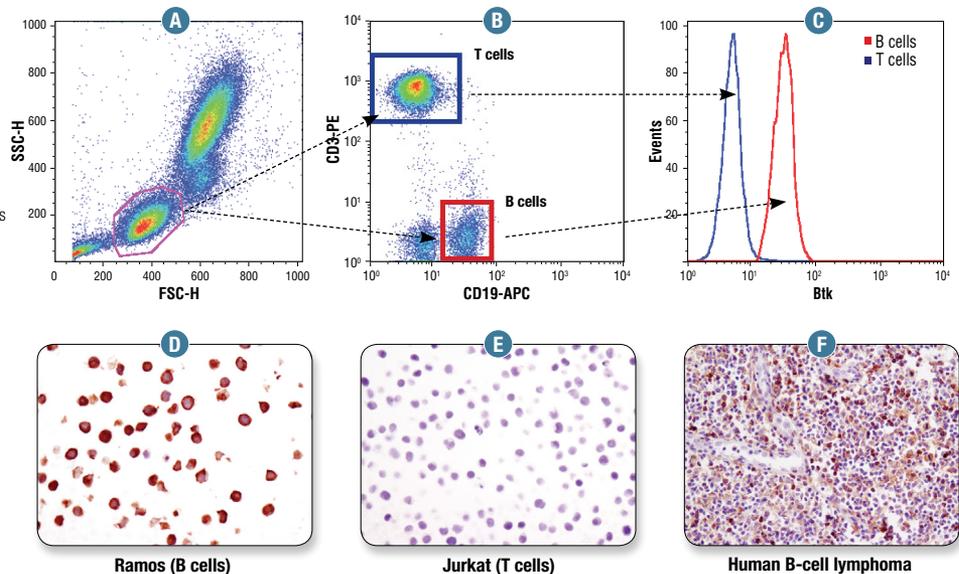
Cell Signaling Technology's dedicated Flow Cytometry group performs rigorous testing in biologically relevant models, ensuring specificity and optimal signal to noise for both purified and conjugated antibodies. Cross-platform validation further confirms antibody specificity, providing the highest quality reagents for flow cytometric analysis of mechanisms underlying cellular signaling.

## Is your antibody specific?

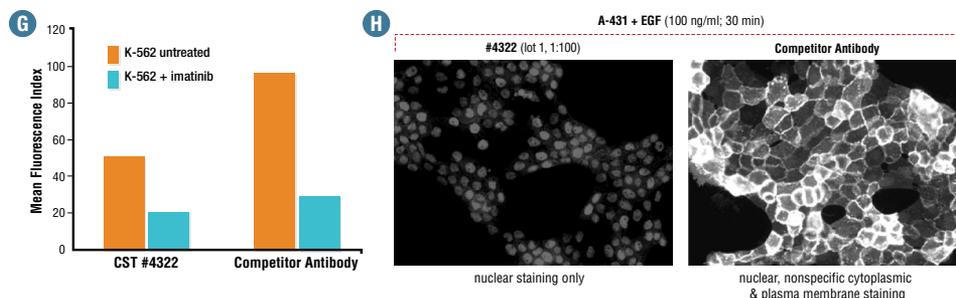
Have more confidence in the specificity of your antibody, because CST has already evaluated each antibody in biologically relevant samples by multiple methods.

### Btk is selectively expressed in B cells.

**Btk (D3H5) Rabbit mAb #8547:** Human whole blood was fixed and permeabilized as outlined in the Cell Signaling Technology Flow Alternate Protocol and stained using #8547. FSC & SSC were used to gate on live cells (A). Samples were co-stained using CD3-PE and CD19-APC to distinguish live T and B cell subpopulations, respectively (B). B (red) and T (blue) cell population gates were applied to a histogram depicting the mean fluorescence intensity of Btk (C). Anti-rabbit IgG (H+L), F(ab)<sub>2</sub> Fragment (Alexa Fluor® 488 Conjugate) #4412 was used as a secondary antibody. IHC analysis of paraffin-embedded cell pellets and tissue (D-F), using #8547.



### Phospho-Stat5 (Tyr694) is localized to the nucleus.



**Phospho-Stat5 (Tyr694) (D47E7) XP® Rabbit mAb #4322:** Flow cytometric analysis to show a lower fold induction of #4322 compared to the competitor's phospho-Stat5 (Tyr694) antibody (G), but fluorescent immunocytochemical staining reveals that the competitor antibody inappropriately stains the cytoplasm and plasma membrane, while #4322 demonstrates only the appropriate nuclear staining (H).

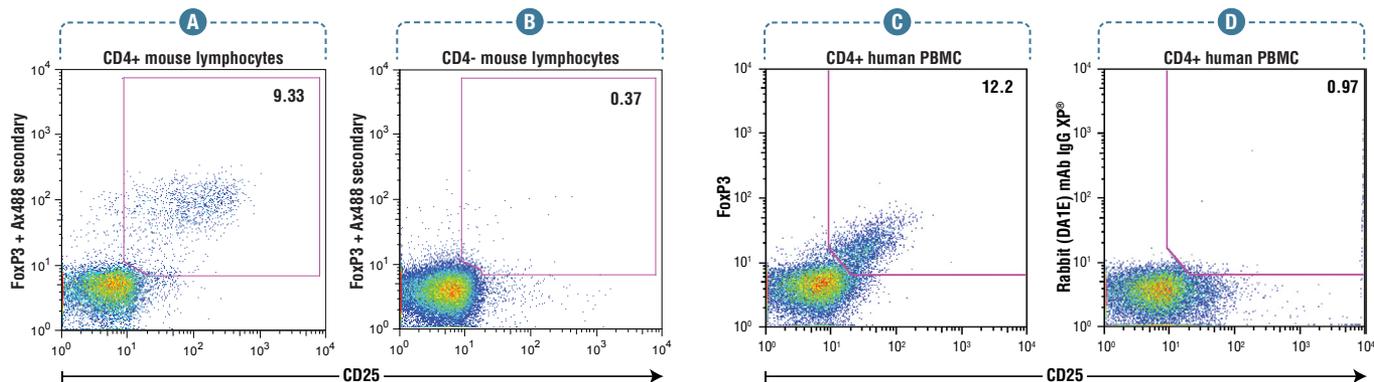


## Is your antibody supported by optimized protocols?

Ensure accurate results in your lab by using protocols optimized to work with CST™ antibodies and your targets.

FoxP3 (D6O8R) XP® Rabbit mAb #12653 and FoxP3 (D6O8C) Rabbit mAb #12632 are each provided with individually optimized protocols, one for analyzing mouse samples and one for analyzing human samples.

The fixation and permeabilization steps of both protocols can be performed using standard lab reagents, so you won't need proprietary buffers and kits to conduct your experiments.

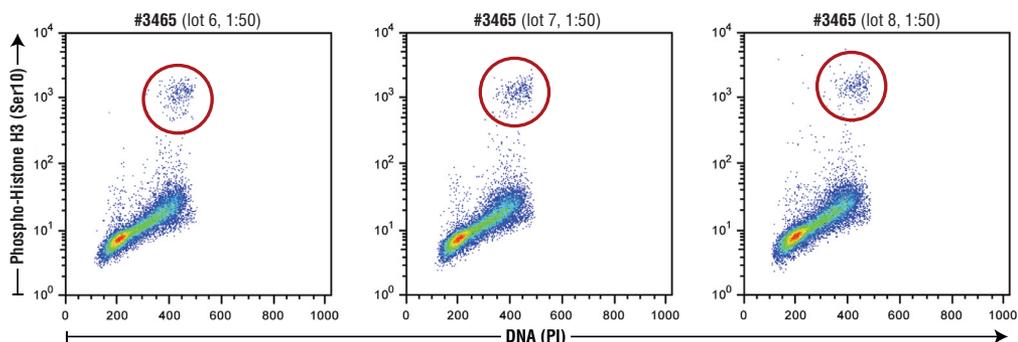


**FoxP3 (D6O8R) XP® Rabbit mAb #12653:** Flow cytometry analysis of murine spleen lymphocytes using a CD25 antibody and #12653. Anti-rabbit IgG (H+L), F(ab')<sub>2</sub> Fragment (Alexa Fluor® 488 Conjugate) #4412 was used as a secondary antibody. Analysis was performed on gated CD4+ lymphocytes (A) and CD4- lymphocytes (B) as an internal negative control. #12653 enables optimal detection of signal intensity and population frequency of FoxP3+/CD25+ T<sub>reg</sub> cells. | **FoxP3 (D6O8C) Rabbit mAb #12632:** Flow cytometry analysis of human peripheral blood mononuclear cells gated on CD4+ lymphocytes, showing FoxP3 expression in CD25+ cells using #12632 (C), and corresponding absence of signal in CD25+ cells using concentration-matched Rabbit (DA1E) mAb IgG XP® Isotype Control #3900 (D). Anti-rabbit IgG (H+L), F(ab')<sub>2</sub> Fragment (Alexa Fluor® 488 Conjugate) #4412 was used as a secondary antibody. FoxP3 (D6O8C) Rabbit mAb #12632 clearly defined the FoxP3+/CD25+ subset with regards to signal intensity and population frequency.

## Is your antibody performing consistently throughout your research?

Ensure reagents are reliable for the life of your project, because CST calibrates every new antibody lot with previous lots to minimize any variation.

**Phospho-Histone H3 (Ser10) (D2C8) XP® Rabbit mAb (Alexa Fluor® 488 Conjugate) #3465:** Flow cytometry analysis of Jurkat cells using multiple lots of #3465 compared to propidium iodide (DNA content). The recommended dilution of #3465 remained 1:50 for all lots compared.



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### ABOUT CST:

Founded by research scientists in 1999, Cell Signaling Technology (CST) is a family-owned company active in applied systems biology research, particularly as it relates to cancer. Understanding the importance of using antibodies with high levels of specificity and consistency, CST scientists produce, validate, and support all of our antibodies in-house.

For a listing of our global locations please visit [www.cellsignal.com/contactus](http://www.cellsignal.com/contactus)