

## BACKGROUND

The CD4-positive helper T cells and CD8-positive cytotoxic T cells comprise the majority of T lymphocytes present in secondary lymphoid organs and are essential for acquired immunity. These two populations are derived from common precursors in the thymus and selected through interaction between their clonal T-cell receptors and major histocompatibility complex molecules. It has been demonstrated that the Runx family of transcription factors, particularly Runx3, is essential for the generation of cytotoxic lineage T cells, whereas the ThPOK/Zbtb7b zinc finger transcription factor that plays a crucial role in the differentiation of the helper lineage. Recent works have implied that a cross-regulation between Runx and ThPOK/Zbtb7b contributes to appropriate thymocyte lineage commitment.<sup>1</sup>

ThPOK/Zbtb7b belongs to a large family of transcription factors that generally act as repressors and are characterized by a carboxyl-terminal DNA binding domain made of multiple zinc fingers (four in ThPOK/Zbtb7b) and an amino-terminal BTB-POZ domain that mediates homodimerization (and possibly heterodimerization). ThPOK/Zbtb7b was identified as essential for CD4 differentiation after a patient quest to identify a spontaneous mutation ("helper deficient") that causes a disruption of mouse CD4 cell development. The culprit proved to be a single amino acid substitution in the second zinc finger of ThPOK/Zbtb7b. In a separate study, ThPOK/Zbtb7b (then named *ckrox*) was identified in a microarray screen for genes up-regulated during positive selection and shown by gain-of-function analyses to inhibit CD8 differentiation and promote CD4 differentiation.<sup>2</sup> Two properties of ThPOK/Zbtb7b deserve emphasis. First, although ThPOK/Zbtb7b is expressed in a wide variety of cells, its expression in the thymus is highly lineage specific: CD4 single-positive (SP) thymocytes (and all CD4 T cells) express ThPOK/Zbtb7b, whereas DP and CD8 SP thymocytes do not. During MHC-II-induced selection, ThPOK/Zbtb7b is up-regulated progressively as thymocytes down-regulate CD8. Second, both loss-of-function and gain-of-function experiments indicate that ThPOK/Zbtb7b affects lineage choice but not positive selection. That is, Thpok-deficient, MHC-II-restricted thymocytes become CD8 instead of CD4 T cells. This analysis, initially performed in helper-deficient mice, was the first genetic demonstration that lineage choice and positive selection were independent, even if contemporaneous, processes.<sup>3</sup>

Although the spotlight has recently been on ThPOK/Zbtb7b, the first transcription factor identified as necessary for CD4 but not CD8 cell differentiation is Gata3, a member of a distinct zinc finger protein family. Loss-of-function analyses using conditional deletion of Gata3 in DP thymocytes or retroviral knockdown short hairpin

RNA transduction, showed that Gata3 is required for the development of CD4 but not CD8 cells. Thus, the current perspective is that ThPOK/Zbtb7b directs CD4 lineage choice whereas Gata3 is needed for the terminal differentiation or survival of CD4 lineage-committed cells. However, it was demonstrated that both transcription factors are required prior to CD4 commitment. Gata3 was required for ThPOK/Zbtb7b expression, whereas ThPOK/Zbtb7b was not necessary for Gata3 expression. Thus, the block caused by Gata3 disruption occurred upstream of that caused by ThPOK/Zbtb7b disruption. Enforced ThPOK/Zbtb7b expression failed to rescue the CD4 differentiation of Gata3-deficient thymocytes, suggesting that Gata3 functions as a key CD4 lineage specification factor, independently from its effects on ThPOK/Zbtb7b expression.<sup>4</sup>

## References

1. He, X. et al: Annu. Rev. Immunol. 28:295-320, 2010
2. Egawa, T.: J. Cell. Biochem. 107:1037-45, 2009
3. Wang, L. & Bosselut, R.: J. Immunol. 183:2903-10, 2009
4. Wang, L. et al: Nat. Immunol. 9:1122-30, 2008

## TECHNICAL INFORMATION

### Source:

ThPOK/Zbtb7b Antibody is a mouse monoclonal antibody raised against purified recombinant human ThPOK/Zbtb7b fragments expressed in *E. coli*.

### Specificity and Sensitivity:

This antibody detects ThPOK/Zbtb7b proteins without cross-reactivity with other related proteins.

**Storage Buffer:** PBS and 30% glycerol

### Storage:

Store at -20°C for at least one year. Store at 4°C for frequent use. Avoid repeated freeze-thaw cycles.

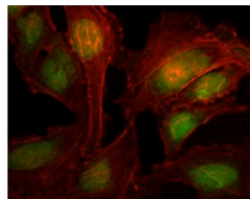
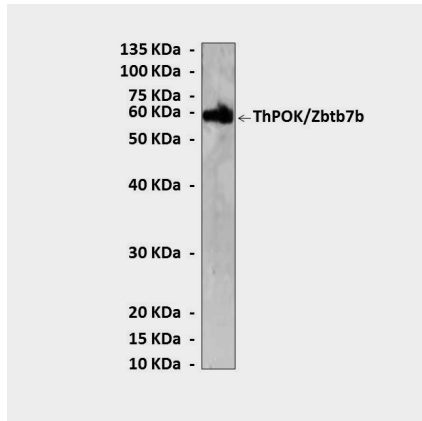
## APPLICATIONS

Application:	*Dilution:
WB	1:1000
IP	n/d
IHC	n/d
ICC	1:50-200
FACS	n/d

\*Optimal dilutions must be determined by end user.



### QUALITY CONTROL DATA



**Top:** Western Blot detection of ThPOK/Zbtb7b proteins in HEK293 cell lysate using ThPOK/Zbtb7b Antibody.  
**Bottom:** this antibody also stains HeLa cells in confocal immunofluorescent analysis (PLZF/ZBTB16 Antibody: Green; Actin filaments: Red).

