AlphaLISA® *SureFire® Ultra™*

Human p-Btk (Tyr223) Detection Kit

Product number: ALSU-PBTK-A500, ALSU-PBTK-A10K, ALSU-PBTK-A50K, ALSU-PBTK-A-HV

Kit specificity:

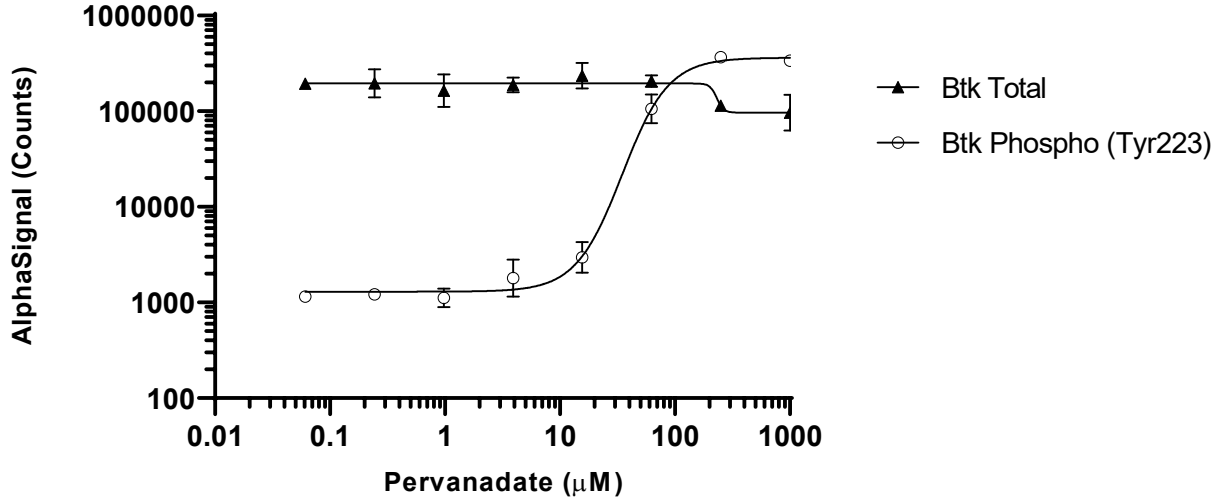
This assay kit contains antibodies which recognize the phospho Tyr223 and a distal epitope on Btk. The protein detected by this kit corresponds to UniProt ID Q06187. BTK is also known as Bruton tyrosine kinase. These antibodies recognize Btk of human origin. Other species should be tested on a case-by-case basis.

Control lysate information:

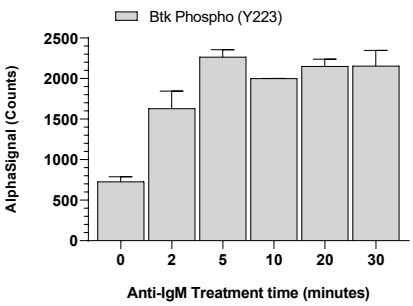
Positive Control Lysate: Prepared from U937 cells cultured in10% FBS containing medium. Cells were harvested, washed with HBSS + 0.1% BSA and adjusted to $2x10^6$ cells/mL then treated with 0.5 mM Pervanadate for 30 minutes. Cells were spun down at 300 RCF and lysed at $1x10^6$ cells/mL with Lysis Buffer.

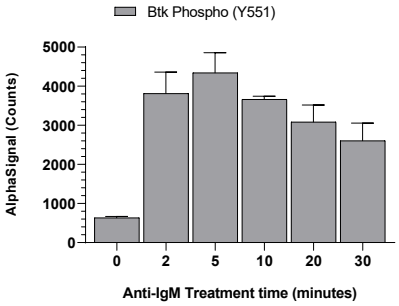
Representative data

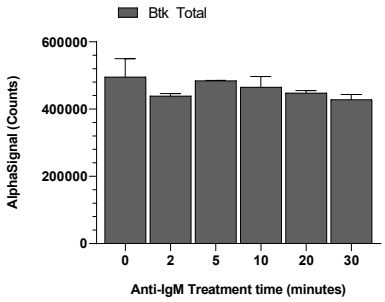
Data obtained with a 2-plate, 2-incubation protocol. U937 cells were washed and resuspended in HBSS + 0.1% BSA and seeded at 200K cells/well in a 96 well plate. Cells were treated with Pervanadate at the indicated concentrations for 30 minutes. Cells were lysed with 5X Lysis Buffer at a final density of 800K cells/mL and assayed separately for Phospho (Tyr223) and Total Btk using respective *SureFire Ultra* kits. Equivalent to approximately 8,000 cells/datapoint.



Data obtained with a 2-plate, 2-incubation protocol. Ramos cells were washed and resuspended in HBSS + 0.1% BSA and seeded at 600K cells/well in a 96 well plate. Cells were treated with 20 µg/mL anti-IgM for varying length of time. Cells were lysed with 5X Lysis Buffer and assayed separately for Phospho (Tyr223, Tyr551) and Total Btk using respective *SureFire Ultra* kits. Equivalent to approximately 24,000 cells/datapoint.







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