RUBY™ – A novel recombinant universal bispecific antibody format for generation of bsAb with outstanding stability, manufacturability and shorter development timelines

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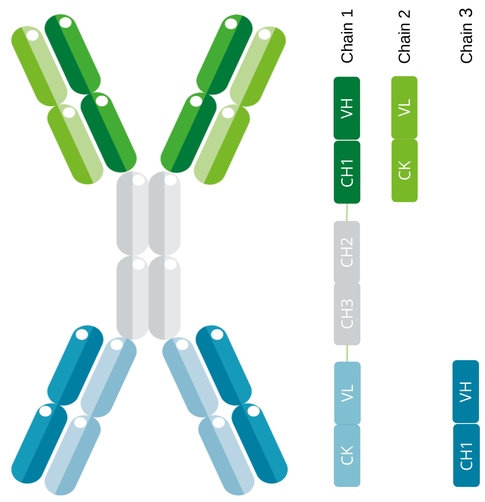
Summary

RUBY™ is novel bispecific antibody (bsAb) format that allows for straightforward generation of new bsAb from any 2 monoclonal antibodies. Its unique architecture differentiates RUBY™ from other formats. RUBY™ bsAb are of the Appended IgG class of bsAb formats. FAb domains are linked through their light chains to the C–terminal end of IgG molecules. This presents several benefits:

1) The lack of single chain fragment (scFv) components allows for a plug-and-play capacity. In contrast to formats containing scFv there is no need for lead optimization and new bsAb can be generated in shorter timelines.
2) Fusion of the appended FAbs via the light chain presents a solution to the common light chain mispairing problem. Products of correct size but with mispaired light chains cannot be produced, making the manufacturing and downstream processing much more straightforward.
3) Presence of Fc domains allows for tailored Fc interactions and possibility to fine tune interaction with FcRn and Fcγ receptors for optimal half-life and function.
4) Tetravalent architecture allows for retained bivalent interaction against each target and more natural antibody-antigen binding kinetics.

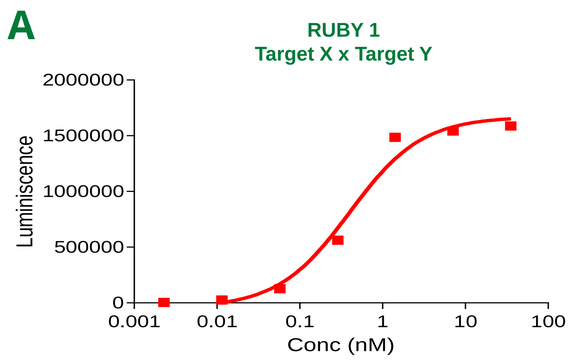
The tetravalent format of RUBY™ makes it a suitable format for applications where effect is gained from clustering or avidity effects, such as co-stimulatory receptor activation were crosslinking often is required for activation, check point blockade, targeting of tumor associated antigens and cytokine blockade. Further on, the RUBY™ format is also highly suited for applications where Fc interactions give benefit, such as MOA involving ADCC, CDC, ADCP and MOA involving cross-linking via Fc, were function can be gained from altering Fc-FcγR interactions and were half-life affects function.

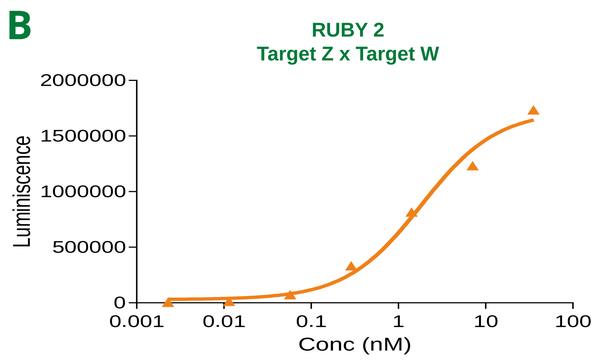


RUBY™ – a plug-and-play technology platform

- ✓ Unique design gives a rapid, simple and cost-effective platform for bsAb generation from any 2 mAbs
- ✓ Fully antibody based
- ✓ No scFv components
- ✓ No mispaired correct size biproducts
- ✓ Fc domain
- ✓ Retained bivalency against each target

Simultaneous binding to both targets and retained affinity

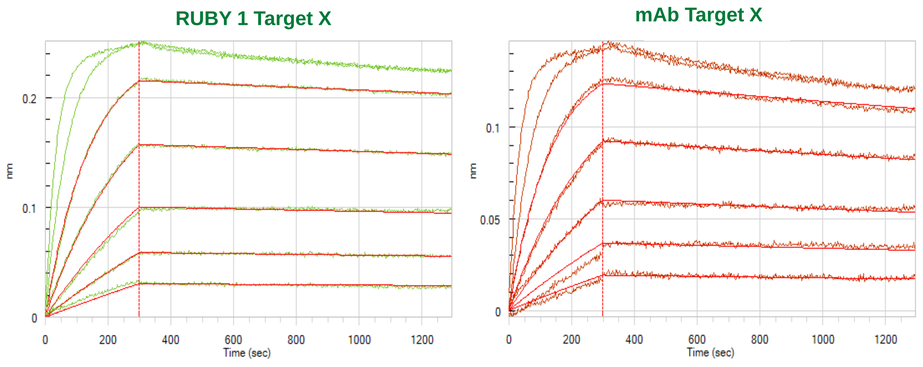




C

Target	Format	K_D (nM)	kon (1/Ms)	koff (1/s)
Target X	RUBY 1	0.02	3E+6	6E-5
	mAb	0.03	4E+6	1E-4
Target Y	RUBY 1	1	1E+6	1E-3
	mAb	1	1E+6	1E-3
Target Z	RUBY 2	0.2	1E+5	3E-5
	mAb	0.2	5E+5	8E-5
Target W	RUBY 2	8	1E+5	1E-3
	scFv	10	2E+5	2E-3

D



The RUBY™ bsAb show simultaneous binding to both its targets, here demonstrated with a dual-ELISA, e.g. capturing with one antigen and detection with the second antigen, as well as unaltered affinity compared to corresponding mAb. A) Dual binding for RUBY 1 against target X and Y, B) Dual binding for RUBY 2 against target Z and W, C) BLI affinity measurements for RUBY 1 and RUBY 2 against respective two targets as measured with Octet, D) Kinetic curves for RUBY 1 and corresponding mAb against target X.

Good manufacturability

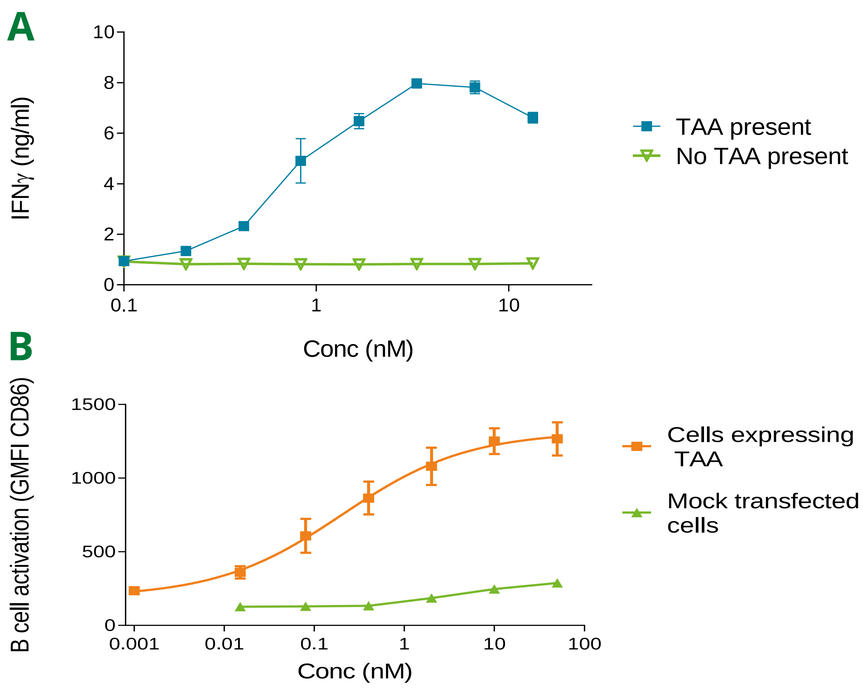
Antibody	Expression system	Average Yield (mg/L)	High molecular weight species (HMWS) (%)
RUBY 1	Expi293	113	2.6
RUBY 2	Expi293	110	1.8
Internal standard (mAb)	Expi293	87	2.3
RUBY 1	ExpiCHO	129	1.8
Internal standard (mAb)	ExpiCHO	135	1.9

The RUBY™ bsAb show good expression yields in transient cultures. The yields are similar to the internal mAb control.

Antibody	25 C, 4 weeks		40 C, 4 weeks		Freeze / thaw, 1 round		Freeze / thaw, 3 rounds	
	Δ% HMWs	Δ% LMWs	Δ% HMWs	Δ% LMWs	Δ% HMWs	Δ% LMWs	Δ% HMWs	Δ% LMWs
RUBY 1	0	0	0	1	0	0	0	0
RUBY 2	0	0	0	2	0	0	0	0

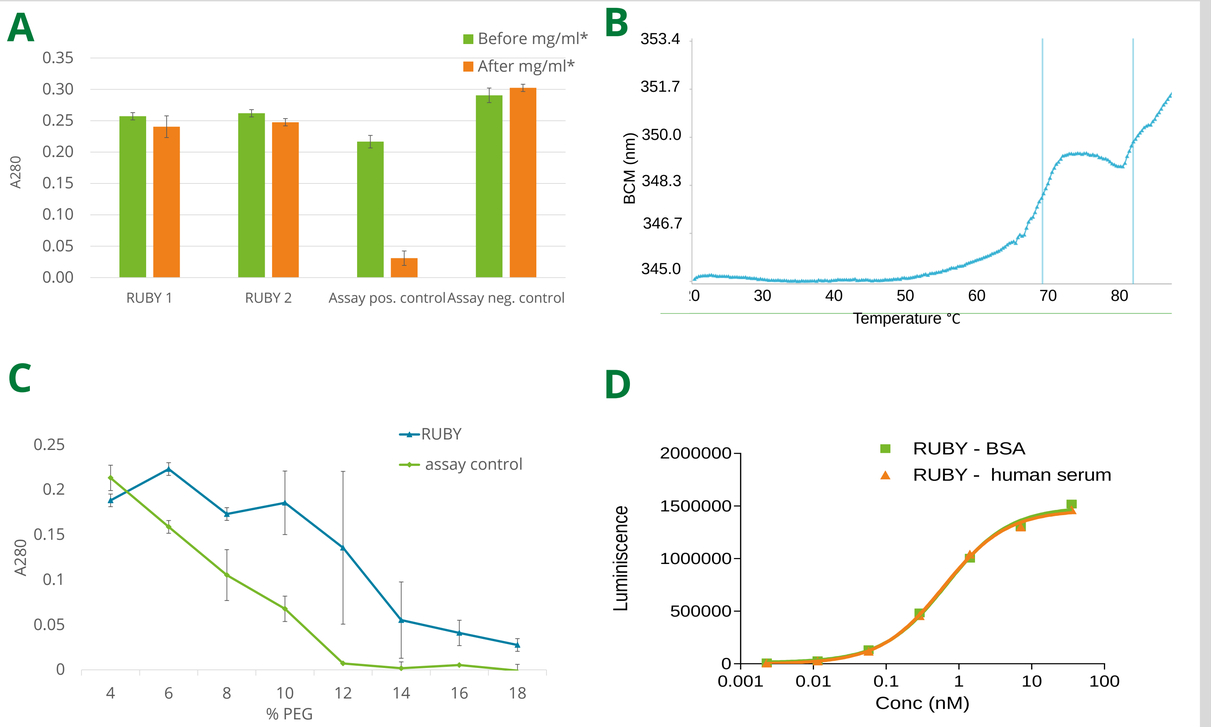
Excellent storage stability is demonstrated for the RUBY™ format. Samples were incubated for 1, 2 and 4 weeks at 2-8 °C, room temperature or 40°C. No or minimal degradation were observed. Even the incubation at 40°C for 4 weeks showed very minimal degradation. Furthermore, no degradation observed following 3 rounds of freeze-thawing.

Effect of RUBY™ bsAb on T-cell and B-cell activation



RUBY™ bsAb show retained function both for T-cell activation and B-cell activation. A) Agonistic function of a RUBY™ bsAb on human CD8 T cells. Dose response dependent IFNγ production (absolute values) by human CD8 T cells from one representative individual donor activated with the bispecific construct in the presence or absence of immobilized TAA, B) Primary human B cells were cultured with titrated antibodies in the presence or absence of TAA expressed on CHO cells. After 2 days, expression of CD86 on B cells was analyzed by FACS. The graph shows pooled results from three donors in one representative experiment of two.

Excellent stability



The RUBY™ format shows excellent stability in regards to shear stress, temperature stability, colloidal stability and serum stability. A) No degradation can be observed after severe agitation treatment in a shear stress evaluation assay, B) The melting temperature and aggregation temperatures are high with Tm > 65 °C as measured by UNcle (Unchained Labs), C) In a colloidal evaluation assay there is no indication of self-binding, D) No difference in binding can be observed after incubation in human serum or PBS with BSA at 37°C for 7 days as measured by dual ELISA, indicating good serum stability.

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