

## **Supporting Information**

### **S9.6 Antibody-Enzyme Conjugates for Detection of DNA-RNA Hybrids**

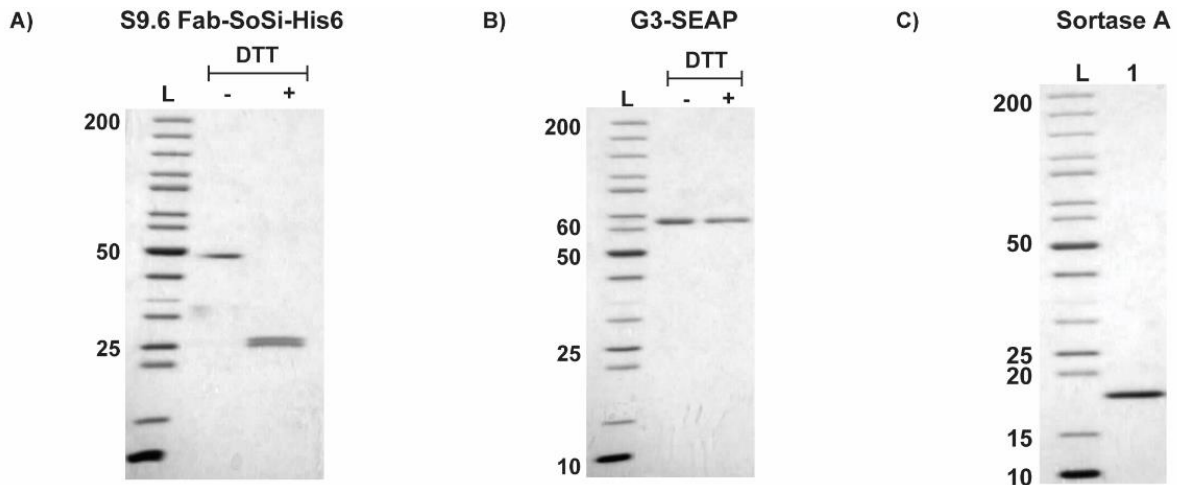
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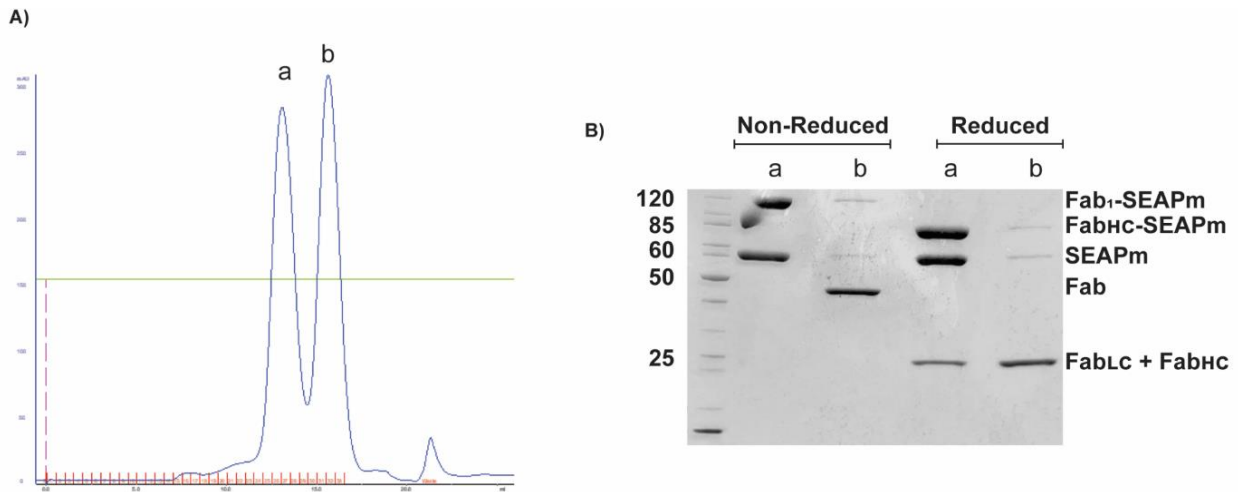
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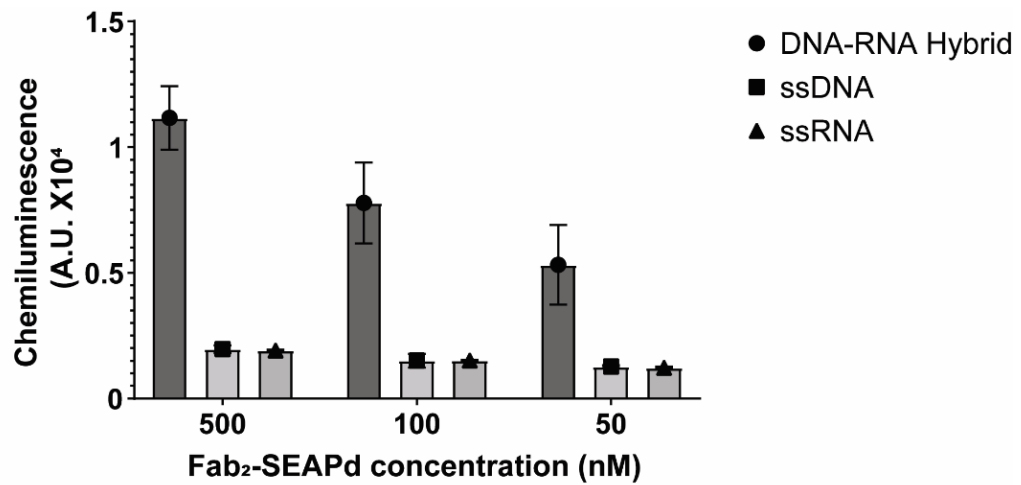
**Supporting Figure:**



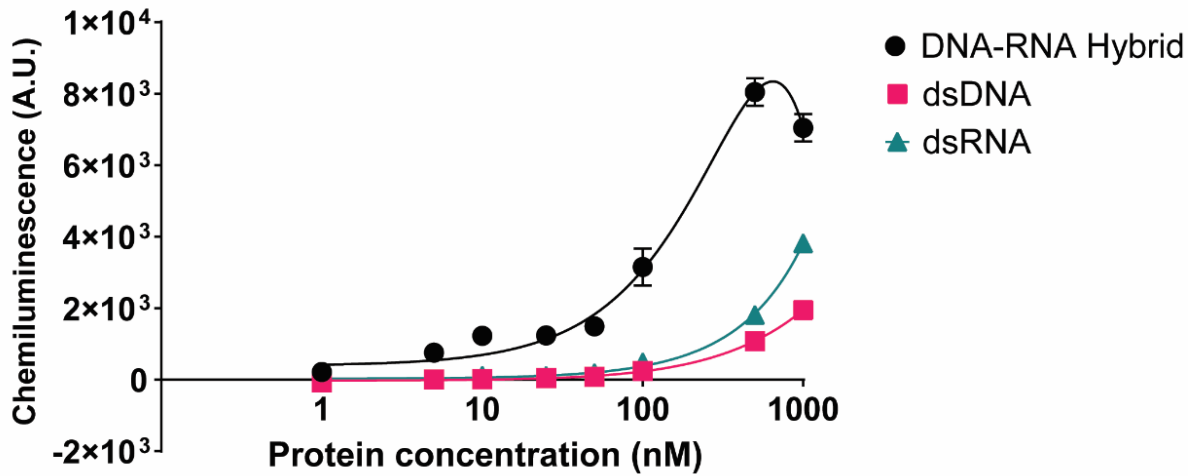
**S1:** (A) Coomassie-stained polyacrylamide gels with a reference ladder [lane L]; (A) disulfide linked S9.6-Fab [49 kDa, lane DTT(-)] and a mixture of the reduced light and heavy chains (24 kDa and 25 kDa, respectively) [lane DTT(+)]; (B) purified G3-SEAP (54 kDa monomer). The G3-SEAP dimer is not disulfide linked. (C) Purified SrtA (lane 1).



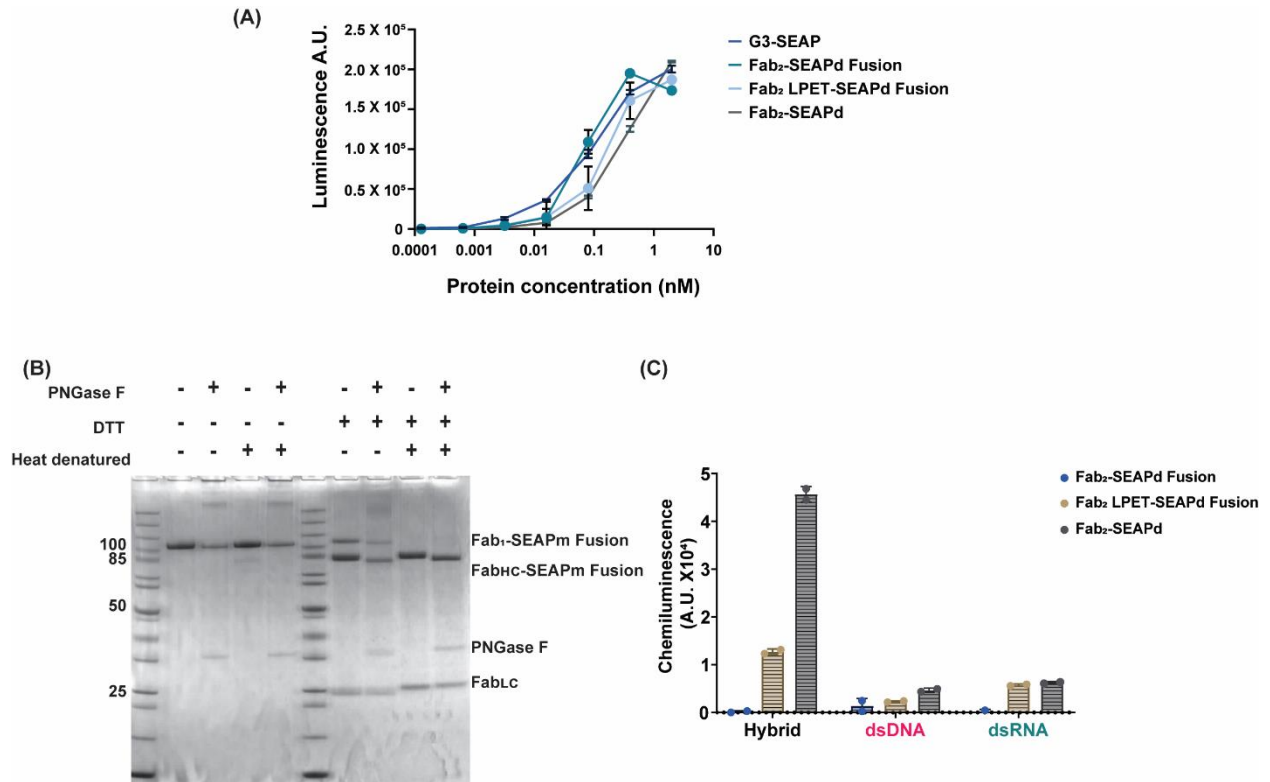
**S2:** (A) Elution profile of proteins from fraction ii of Fig. 2A purified through size-exclusion Superdex-200 column. The two peaks marked as “a” and “b” were collected. (B) Peak “a” has Fab<sub>L</sub>-SEAP<sub>m</sub> (114 kDa) and possibly some free SEAP<sub>d</sub> (seen as denatured SEAP<sub>m</sub> at 54 kDa in non-reduced lane). The reduced lane confirms site specific conjugation of SEAP to Fab<sub>Hc</sub> (seen as Fab<sub>Hc</sub>-SEAP<sub>m</sub> at 84 kDa). Peak “b” non-reduced and reduced lanes show the presence of disulfide linked S9.6 Fab (seen as Fab<sub>Lc</sub> + Fab<sub>Hc</sub> at 25 kDa).



**S3:** Specific binding of 10 pmole of DNA-RNA hybrid, ssDNA and ssRNA with 500, 100 or 50 pmole S9.6 Fab<sub>2</sub>-SEAPd. in 100  $\mu$ L of reaction measured in chemiluminescent units (A.U.)



**S4: The HC-S ELISA using S9.6 Fab<sub>2</sub>-SEAPd genetic fusion protein:** Specific binding and identification of DNA-RNA hybrid with S9.6 Fab-SEAP genetic fusion protein is quantified by chemiluminescence (A.U.). Note: the S9.6 Fab-SEAP genetic fusion protein could non-specifically detect the dsDNA and dsRNA after a high concentration of 1  $\mu$ M.



**S5: Investigation of difference in activity between sortase A conjugated and genetic fusion protein of S9.6 Fab-SEAP.** (A) SEAP enzyme catalytic activity in G3-SEAP and variants of Fab<sub>2</sub>-SEAPd proteins. The activity was measured in chemiluminescence units (A.U.) using mentioned concentrations of individual proteins. (B) Mass shift observed in deglycosylated S9.6 Fab<sub>2</sub>-SEAPd genetic fusion protein. The mass shift was only observed in the HC-SEAP fusion polypeptide. Samples with DTT but without PNGase F or heat show incomplete reduction. (C) Experiment done as in Fig. 5C but with deglycosylated S9.6 Fab-LPET-SEAP genetic fusion and Fab<sub>2</sub>-SEAPd proteins.

**Supporting File 1:**

>G3-SEAP [With mouse N-terminal IgG signal sequence, bold and underlined]

**MGWSCILFLVATATGVHS**GGGIIPVEEENPDFWNREAAEALGAAKKLQPAQTAAKNLIIFLGD  
GMGVSTVTAARILKGQKKDKLGPEIPLAMDRFPYVALSKTYNVDKHVPDSGATATAYL CGVKG  
NFQTIGLSAAARFNQCNTTRGNEVISVMNRAKKAGKSVGVTTRVQHASPAGTYAHTVNRNW  
YSDADVPASARQEGCQDIATQLISNMDIDVILGGGRKYMFRMGTPDPEYPDDYSQGGTRLDGKN  
LVQEWLAKRQGARYVWNRTELMQASLDPSVTHLMGLFEPGDMKYEIHRDSTLDPSLMEMTEA  
ALRLLSRNPRGFVVEGGRIDHGHESRAYRALTETIMFDDAIERAGQLTSEEDTSLVTADHSH  
VFSFGGYPLRGSSIFGLAPGKARDRKAYTVLLYGNGPGYVLKDGARPDVTESESGSPEYRQQA  
VPLDEETHAGEDVAVFARGPQAHLVHGVQEQTFAHVMAFAACLEPYTACDLAPPAGTTDAAH  
PG\*

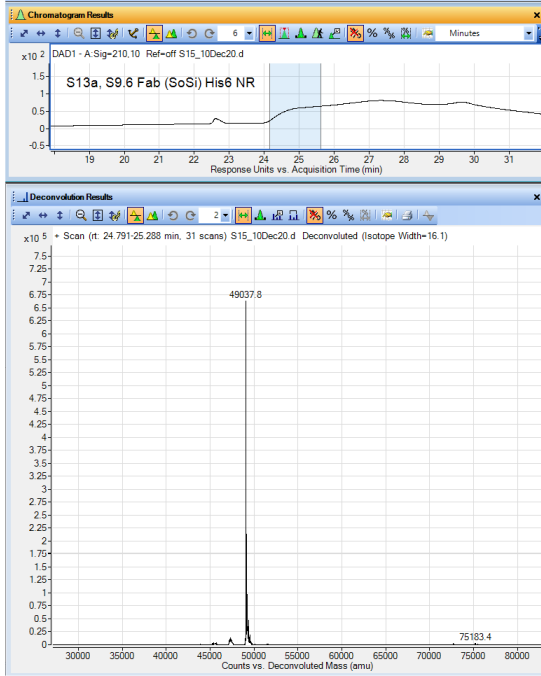
>G3-SEAP [secreted G3-SEAP with N-terminal GGG, red]

GGGIIPVEEENPDFWNREAAEALGAAKKLQPAQTAAKNLIIFLGDGMGVSTVTAARILKGQKKD  
KLGPEIPLAMDRFPYVALSKTYNVDKHVPDSGATATAYL CGVKGNFQTIGLSAAARFNQCNTTR  
GNEVISVMNRAKKAGKSVGVTTRVQHASPAGTYAHTVNRNWYSDADVPASARQEGCQDIA  
TQLISNMDIDVILGGGRKYMFRMGTPDPEYPDDYSQGGTRLDGKNLVQEWLAKRQGARYVWN  
RTELMQASLDPSVTHLMGLFEPGDMKYEIHRDSTLDPSLMEMTEAALRLLSRNPRGFVVEGG  
RIDHGHESRAYRALTETIMFDDAIERAGQLTSEEDTSLVTADHSHVFSFGGYPLRGSSIFGLAP  
GKARDRKAYTVLLYGNGPGYVLKDGARPDVTESESGSPEYRQQA VPLDEETHAGEDVAVFAR  
GPQAHLVHGVQEQTFAHVMAFAACLEPYTACDLAPPAGTTDAAH PG\*

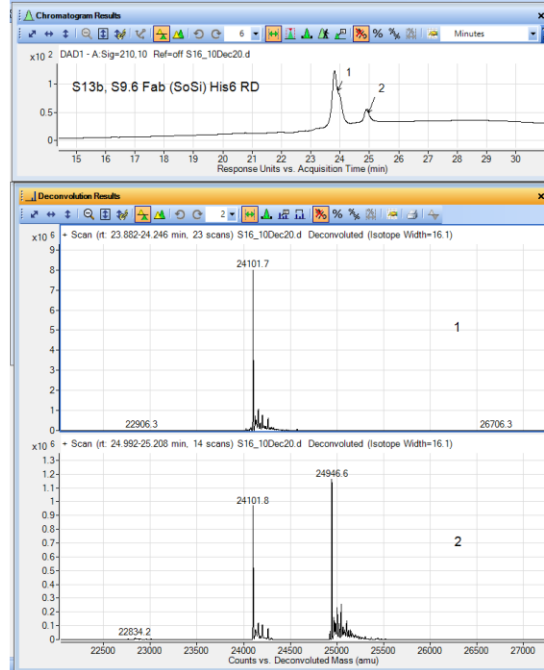
## Supporting File 2:

### (A) Intact mass analysis of S9.6-Fab SoSi-His6:

Non-reduced S9.6-Fab SoSi-6His

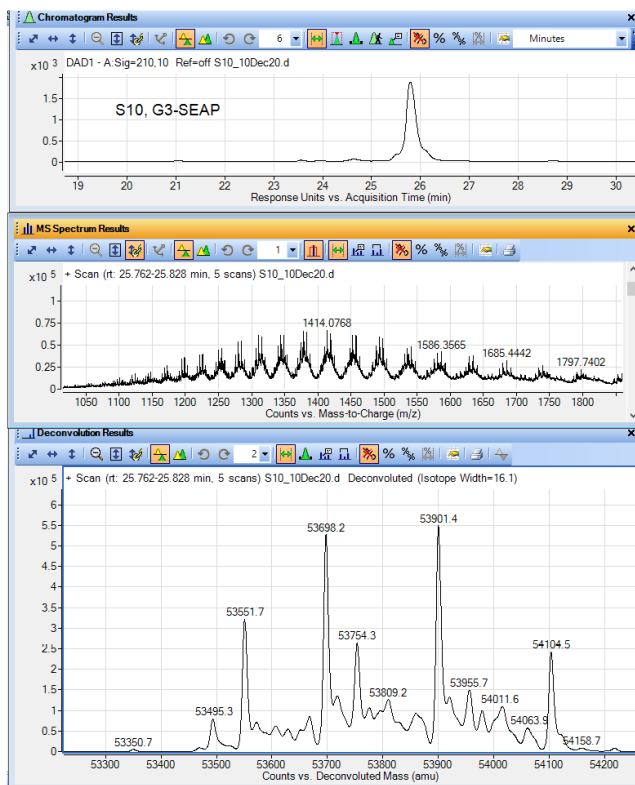


Reduced S9.6-Fab SoSi-His6



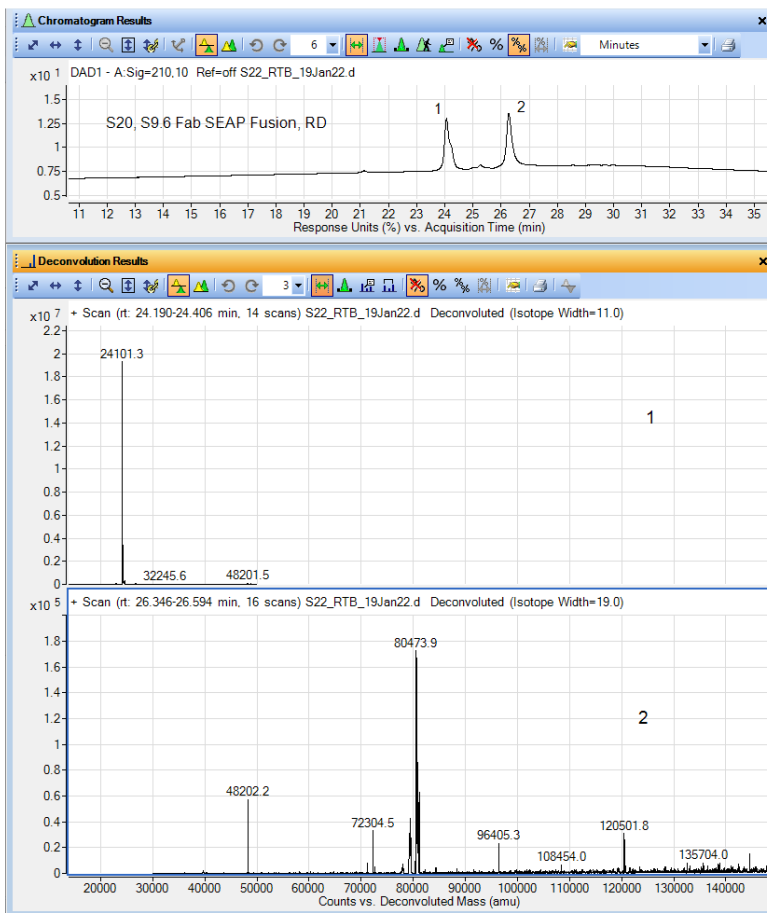
Protein	Calculated Mass	Observed Mass	$\Delta$ Mass	Possible modification
S9.6 Fab (SoSi) His6 Non-reduced Peak at 24.79 min – 25.28 min	49046.7	49037.8	-8.9	None
S9.6 Fab (SoSi) His6 reduced Peak 1 at 23-24 min	24100.8	24101.7	0.91	None
S9.6 Fab (SoSi) His6 reduced Peak 2 at 25 min	24100.8	24101.8	1.01	None

**(B) Intact mass analysis of GGG-SEAP (G3-SEAP):**



Polypeptide	Calculated Mass	Observed Mass	$\Delta$ Mass	Possible modification
GGG-SEAP	53349.93	53350.7	0.77	Non-glycosylated
	53349.93	53495.3	145.37	Minor component
	53349.93	53551.7	201.77	Minor component
	53349.93	53698	348.07	Possible glycosylation
	53349.93	53754	404.07	Minor component
	53349.93	53901	551.07	Possible glycosylation
	53349.93	54104	754.07	Minor component

**(C) Intact mass analysis of S9.6-Fab-SEAP fusion:**



Polypeptide	Calculated Mass	Observed Mass	$\Delta$ Mass	Possible modification
HC	24881.91	24882.20	0.29	None
LC	24100.79	24101.30	0.51	None
HC-GGG-SEAP	77723.38	80475.20	2751.82	Glycosylation