

# Supplementary material

## A1 - Preliminary experiments

In order to be able to select additives, which have an impact on the mAb solution stability, dynamic light scattering (DLS) measurements were carried out. With the help of the DLS measurements and the Stokes-Einstein equation, the hydrodynamic radius ( $r_h$ ) of the antibody was determined as well as higher molecular weight species detected. The increase of the  $r_h$  of the mAb and the detection of higher molecular weight species indicate a colloidal destabilization of the mAb<sup>1,2</sup>. A reduction of  $r_h$  as well as the complete elimination of higher molecular weight species indicate a stabilizing character of the tested additive. Additives with a visual pH and concentration dependent impact will be selected for the actual study. First, the reference value of the antibody without additives was determined at pH 5, 7 and 9. The additive was then added to this solution in the lowest concentration and measured after an hour of waiting time. This procedure was repeated with increasing additive concentration.

*Table 1: Type and concentration of additives used for pre-tests.*

additive	$C_{\text{additive}}$ [M]
glycine	0.05/0.1/0.15/0.2/0.25/0.3
glycerol	0.01/0.02/0.03/0.04
D-sorbitol	0.02/0.04/0.06/0.08
PEG4000	0.002/0.004/0.006/0.008/0.01
Na <sub>2</sub> SO <sub>4</sub>	0.06/0.08/0.1/0.12/0.14/0.16
Nal	0.025/0.05/0.1/0.15/0.2/0.4/0.8

Glycine decreased the  $r_h$  of the mAb at pH 7 and pH 9 with increasing glycine concentration. At pH 5 the  $r_h$  decreased with the addition of glycine but was not glycine concentration dependent. With glycine in solution no higher molecular weight species could be detected. Glycerol and D-sorbitol had a comparable impact on the antibody tested. At pH 5 and pH 7 a slight increase of the  $r_h$  of the mAb was observed whereas at pH 7 a slight decrease appeared. The impact of osmolyte concentration was not as pronounced as for glycine.

PEG4000 had a strong increasing impact on the  $r_h$  of the mAb and also on the development of higher molecular weight species for all pH values tested. The effect was mostly pronounced at higher PEG concentrations and at pH 7.

With the addition of  $\text{Na}_2\text{SO}_4$  the  $r_h$  of the mAb molecules increased. This effect was  $\text{Na}_2\text{SO}_4$  concentration dependent at all pH values tested. NaI had an increasing impact on the  $r_h$  at pH 5 comparable to  $\text{Na}_2\text{SO}_4$ . At pH 7 instead low NaI concentrations had a strong increasing impact on the  $r_h$  of the mAb. This effect decreased with increasing NaI concentration to a marginal small effect at the maximal NaI concentration (800 mM) tested. At pH 9 the impact of NaI as an additive on the mAb  $r_h$  could not be tested due to immediate precipitation.

### A3 – Scoring of the analytical results

Table 2a: Results of the thermal stability test. Listed are average values (duplicate measurement) of  $T_{m1}$ ,  $T_{m2}$  and  $T_{agg}$  of all samples tested.

Thermal stability results 1						
pH	mAb conc.	additive	additive conc.	$T_{m1}$	$T_{m2}$	$T_{agg}$
[ ]	[mg/ml]		[ ]	[°C]	[°C]	[°C]
5	120	no add	-	72.83	-	71.03
5	180	no add	-	66.80	-	73.40
7	1	no add	-	66.87	54.77	70.53
7	5	no add	-	66.63	76.43	71.80
7	15	no add	-	66.70	75.83	70.90
7	30	no add	-	66.43	75.03	70.83
7	50	no add	-	65.40	74.30	70.60
7	70	no add	-	74.93	-	71.13
7	100	no add	-	74.40	-	70.83
7	120	no add	-	75.77	-	70.07
7	180	no add	-	77.20	-	72.20
9	100	no add	-	74.57	-	67.90
9	120	no add	-	75.33	-	68.33
9	180	no add	-	75.90	-	69.43
[ ]	[mg/ml]		[ mM]	[°C]	[°C]	[°C]
5	120	glycine	50	70.37	-	72.40
5	120	glycine	100	71.63	-	72.60
5	120	glycine	150	75.87	-	72.70
5	180	glycine	50	67.97	-	73.43
5	180	glycine	100	67.23	-	73.50
5	180	glycine	150	67.97	-	73.53
7	120	glycine	50	76.40	-	71.20
7	120	glycine	100	76.30	-	70.67
7	120	glycine	150	76.60	-	70.60
7	180	glycine	50	78.20	-	70.20
7	180	glycine	100	78.40	-	70.10
7	180	glycine	150	79.10	-	70.10
9	120	glycine	50	75.83	-	69.80
9	120	glycine	100	76.23	-	68.63
9	120	glycine	150	76.30	-	69.30
9	180	glycine	50	76.10	-	69.93
9	180	glycine	100	76.53	-	69.73
9	180	glycine	150	76.63	-	70.33

Table 2b: Results of the thermal stability test. Listed are average values (duplicate measurement) of  $T_{m1}$ ,  $T_{m2}$  and  $T_{agg}$  of all samples tested.

Thermal stability results 2						
pH	mAb conc.	additive	additive conc.	$T_{m1}$	$T_{m2}$	$T_{agg}$
[ ]	[mg/ml]		[(m/V)%]	[°C]	[°C]	[°C]
9	120	PEG4000	0.4	68.30	-	67.17
9	120	PEG4000	1.2	68.47	-	65.60
9	120	PEG4000	2.0	72.33	-	65.10
[ ]	[mg/ml]		[mM]	[°C]	[°C]	[°C]
7	120	Na <sub>2</sub> SO <sub>4</sub>	100	76.67	-	70.57
7	120	Na <sub>2</sub> SO <sub>4</sub>	140	76.65	-	70.15
7	120	Na <sub>2</sub> SO <sub>4</sub>	160	76.85	-	60.55
7	180	Na <sub>2</sub> SO <sub>4</sub>	100	77.05	-	71.60
7	180	Na <sub>2</sub> SO <sub>4</sub>	140	77.55	-	71.35
7	180	Na <sub>2</sub> SO <sub>4</sub>	160	77.76	-	71.77

### A3 – Scoring of the analytical results

Table 3: Scoring values of the analytical methods applied. The raw analytical results were normalized and a scoring from 0 to 10 defined. Whereby 10 is a result indicating a high colloidal stability and 0 an analytical result indicating a weak solution stability of the screened mAb.

scoring - no additive							
analytical method	parameter	pH 5		pH 7		pH 9	
		120 mg/ml	180 mg/ml	120 mg/ml	180 mg/ml	120 mg/ml	180 mg/ml
SEC	monomer	10.00	8.64	1.47	0.15	3.53	1.38
thermal stability	T <sub>agg</sub>	5.20	9.75	3.34	7.44	0.01	2.12
viscosity	η	9.31	1.79	9.93	4.56	9.92	4.69
charge	ζ-potential	10.00	10.00	2.98	2.98	0.11	0.11
viscoelasticity	ω <sub>co</sub>	10.00	7.47	3.93	0.55	5.72	2.61
AVERAGE VALUE		8.90	7.53	4.33	3.14	3.86	2.18

scoring - glycine							
analytical method	parameter	pH 5		pH 7		pH 9	
		120 mg/ml	180 mg/ml	120 mg/ml	180 mg/ml	120 mg/ml	180 mg/ml
SEC	monomer	9.84	9.14	3.88	4.36	2.83	3.21
thermal stability	T <sub>agg</sub>	8.40	10.01	4.37	6.29	1.87	3.85
viscosity	η	9.12	2.06	10.00	6.33	9.89	4.31
charge	ζ-potential	0.44	0.44	3.08	3.08	0.55	0.55
viscoelasticity	ω <sub>co</sub>	8.55	4.37	3.30	0.70	7.03	3.38
AVERAGE VALUE		7.27	5.21	4.92	4.15	4.43	3.06

scoring - PEG4000							
analytical method	parameter	pH 5		pH 7		pH 9	
		120 mg/ml	180 mg/ml	120 mg/ml	180 mg/ml	120 mg/ml	180 mg/ml
SEC	monomer	-	-	-	-	0.48	0.05
thermal stability	T <sub>agg</sub>	-	-	-	-	7.63	-
viscosity	η	-	-	-	-	8.68	0.45
charge	ζ-potential	1.75	1.75	0.52	0.52	0.61	0.61
viscoelasticity	ω <sub>co</sub>	9.31	6.43	4.10	2.10	5.03	0.00
AVERAGE VALUE		5.53	4.09	2.31	1.31	4.49	0.15

## References

1. Hanlon A., Larkin M., and Reddick R. Free-solution, label-free protein-protein interactions characterized by dynamic light scattering. *Biophysical journal*, 98(2):297-304, 2010. doi:10.1016/j.bpj.2009.09.061.
2. Bauer K. C., Göbel M., Schwab M.-L., Schermeyer M.-T., and Hubbuch J. Concentration dependent changes in apparent diffusion coefficients as indicator for colloidal stability of protein solutions. *International Journal of Pharmaceutics*, 511(1):276-287, 2016. doi: 10.1016/j.ijpharm.2016.07.007.