

# Nimble Junior™

## High Throughput Evaluation of Protein Solubility

# Nimble Junior™

## Quick Automated Assessment of Protein Solubility on a Microplate

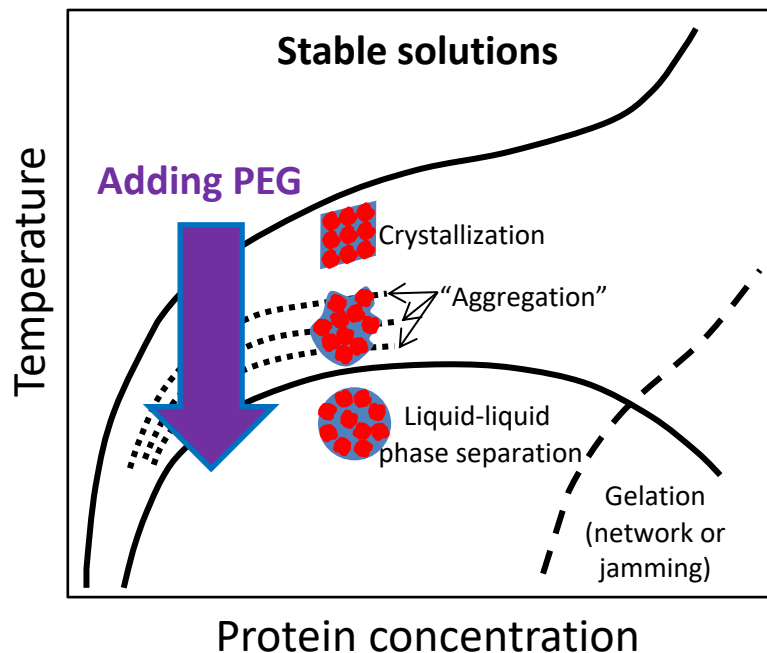
Same day optimal buffer/pH identification **using low amount of protein**

- Fast but rigorous assessment of protein solubility
- Ready-to-use formulation matrices
- When protein sample is limited
- Easy setup, automated measurement and data analysis



# How Nimble Junior™ Works

- Nimble Junior evaluates protein solubility through protein precipitation from **liquid-liquid phase separation (LLPS)** induced by polyethylene glycol (PEG).
- PEG-induced LLPS method is an established and well-recognized thermodynamic method.



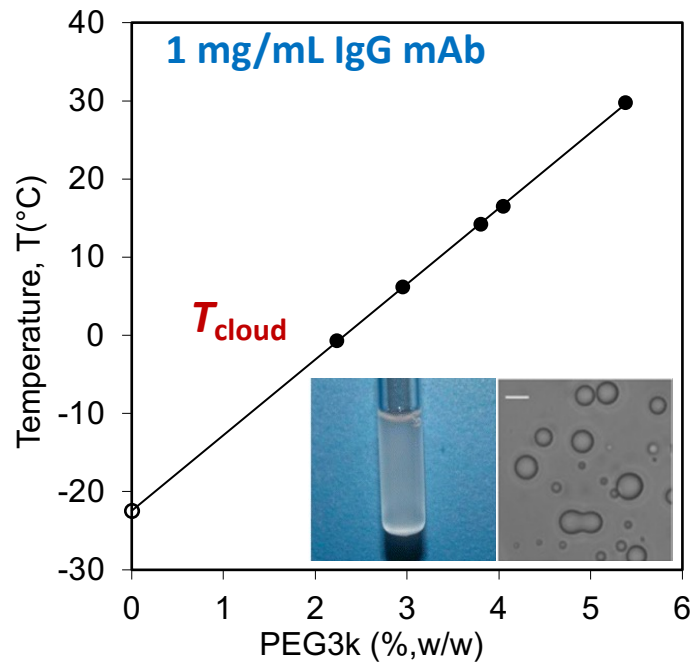
Wang et. al. *J. Chem. Phys.*, 139, 121904 (2013)

Our tech is validated by these peer-reviewed non-sponsored publications:

- Scannell et. al. *Pharm. Res.*, 38(11), 1947 (2021)  
(Eli. Lily)
- Latypov and Wang, *Methods in Mol. Bio.*, 2039, 39 (2019)  
(Sanofi)
- Chai et. al., *mAbs*, 11(4), 747 (2019)  
(Eli. Lily)
- Wang et. al., *Langmuir*, 33, 7715 (2017)  
(Hoffmann-La Roche)
- Rowe et. al. *Biophysical J.* 113, 1750 (2017)  
(MedImmune)
- Wang et. al. *Mol. Pharm.*, 11(5), 1391 (2014)  
(Amgen)
- Li et. al. *Protein Sci.*, 22(8), 1118 (2013)  
(Pfizer)
- Gibson et. al. *J. Pharm. Sci.*, 100(3), 1009 (2011)  
(Johnson & Johnson)

# LLPS $T_{\text{cloud}}$ and Solubility

- The liquid-liquid phase separation temperature  $T_{\text{cloud}}$  ranks protein solubility in different formulations.



Wang et. al. *J. Chem. Phys.*, 139, 121904 (2013)

- A higher  $T_{\text{cloud}}$  indicates lower solubility, which correlates with various issues including crystallization, aggregation, gelation, and liquid-liquid phase separation in the formulation.

# Pre-Formulation Screening

- Nimble Junior™ runs experiments and analyzes data automatically.
- **10 minutes** hands-on time, **1.5 hours** experiment runtime:
  - Step One           **ADD** kit reagents and Incubate 8 min
  - Step Two           **ADD** your protein, **MIX**, and Click **RUN**
  - Step Three        **LEAVE** the rest to Nimble Junior, Get your results in 1.5 hours
- Select a reagent zone in the kit plate that is appropriate for the protein to be evaluated.
- For the Protein stock solution
  - Add a stock protein solution to the kit reagents to achieve a final concentration of  $\leq 1$  mg/ml.
  - Same size proteins and same protein concentration need to be used for all samples in one experiment.

# Formulation Kits\*

\*Patent Pending

ProStabilis offers pre-made  
**Formulation Kits.**

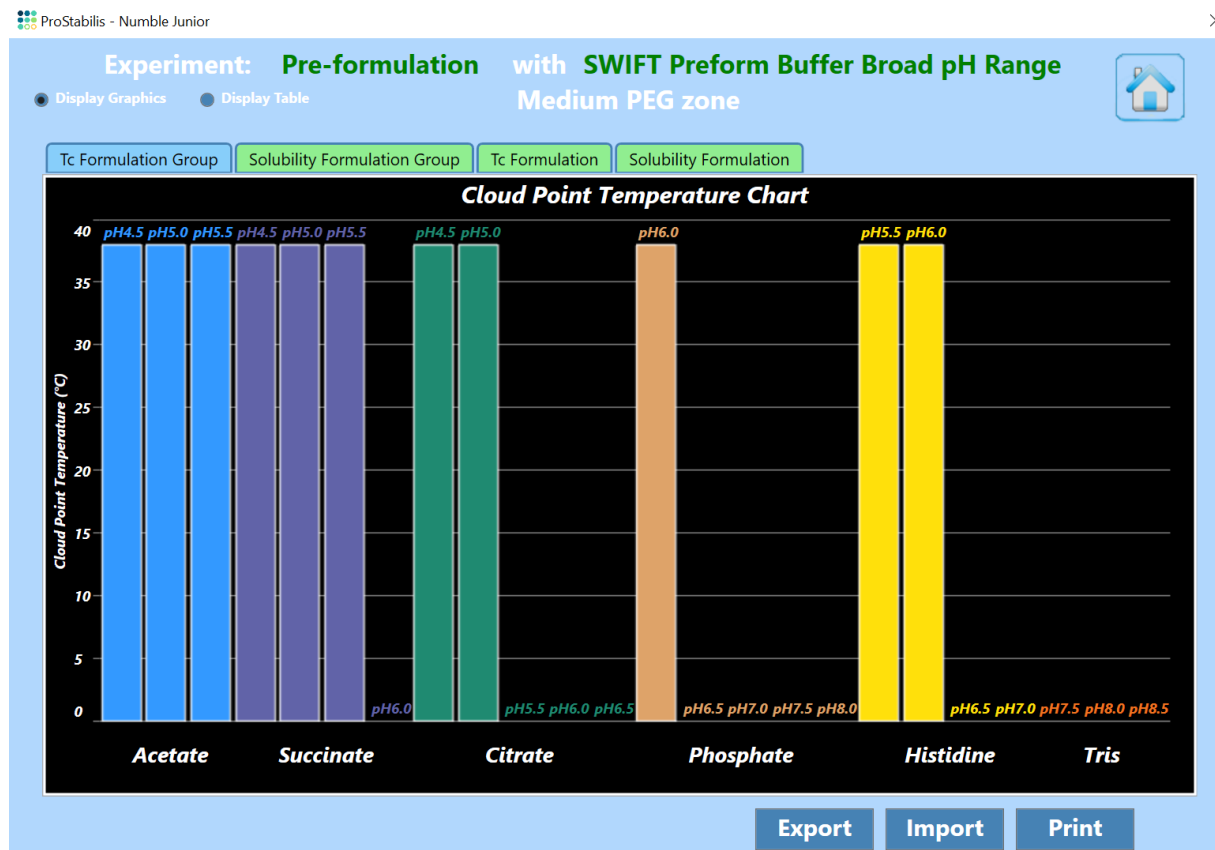


Example Kit Selection:

- **SWIFT™ Buffer/Broad pH Range Kit (Cat. # PS2-PFK-01)**
  - With 6 most popular buffers covering 24 formulations from pH 4.5 to 8.5
    - Acetate 4.5 – 5.5
    - Succinate 4.5 – 6.0
    - Citrate 4.5 – 6.5
    - Histidine 5.5 – 7.0
    - Phosphate 6.0 – 8.0
    - Tris 7.5 – 8.5
  - Different reagent zones in one kit plate allow optimization for a specific protein, and for assessment across poorly and highly soluble proteins.
  - 1.5 mL of formulation reagent provides for 15 experiments.

# Case I: Buffer/pH Screening for BSA

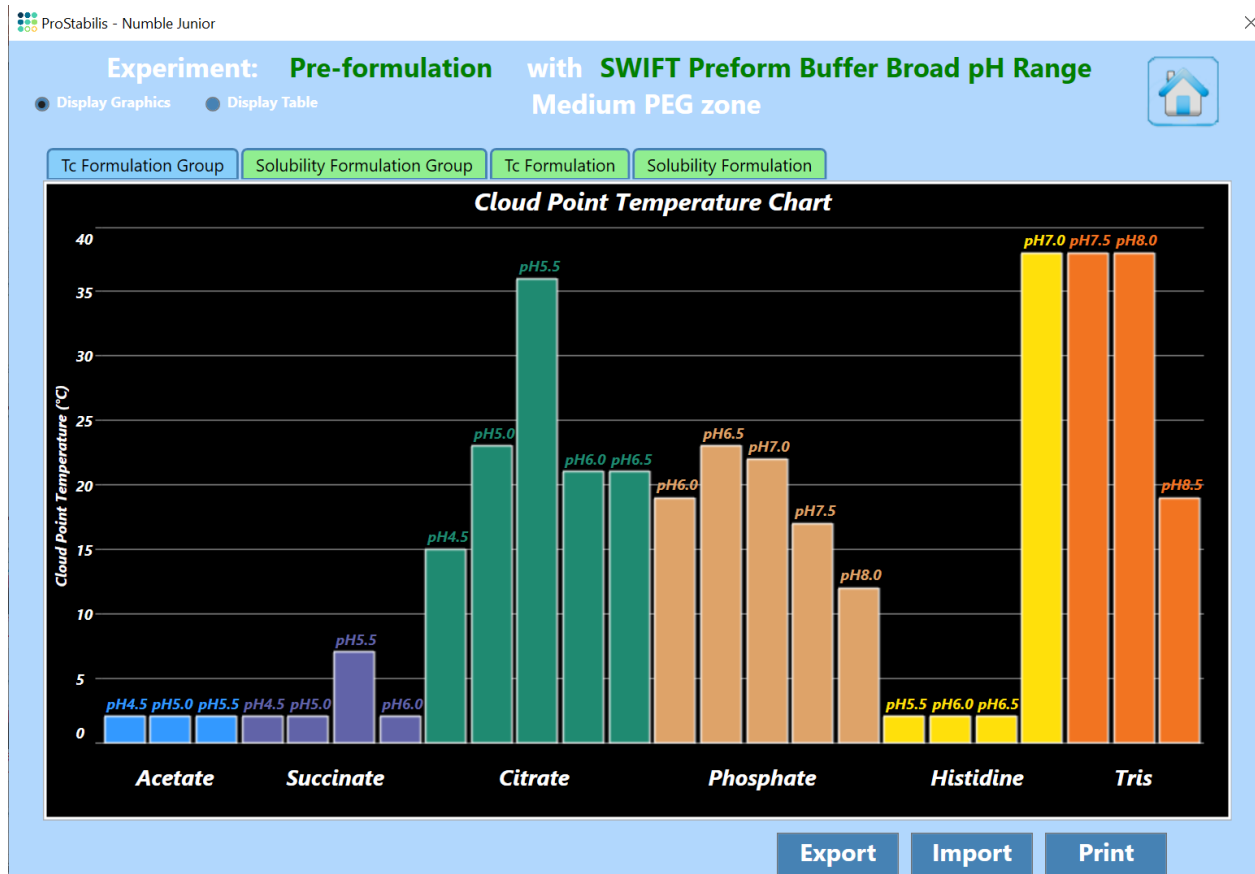
Nimble Junior™ ranks protein solubility by  $T_{\text{cloud}}$ : Lower  $T_{\text{cloud}}$ ,  $\Rightarrow$  Higher solubility



- The isoelectric point of Bovine Serum Albumin (BSA) is  $\sim 5$ .
- Nimble Junior detects that BSA has higher solubility at  $\text{pH} > 6.0$  in all buffers.
- At  $\text{pH} 6.0$ , BSA is more soluble in succinate and citrate than in phosphate or histidine. At  $\text{pH} 5.5$ , BSA is more soluble in citrate than in succinate.

# Case II: Buffer/pH Screening for a mAb

Formulation buffer screening for a pharmaceutical mAb (pI = 7)

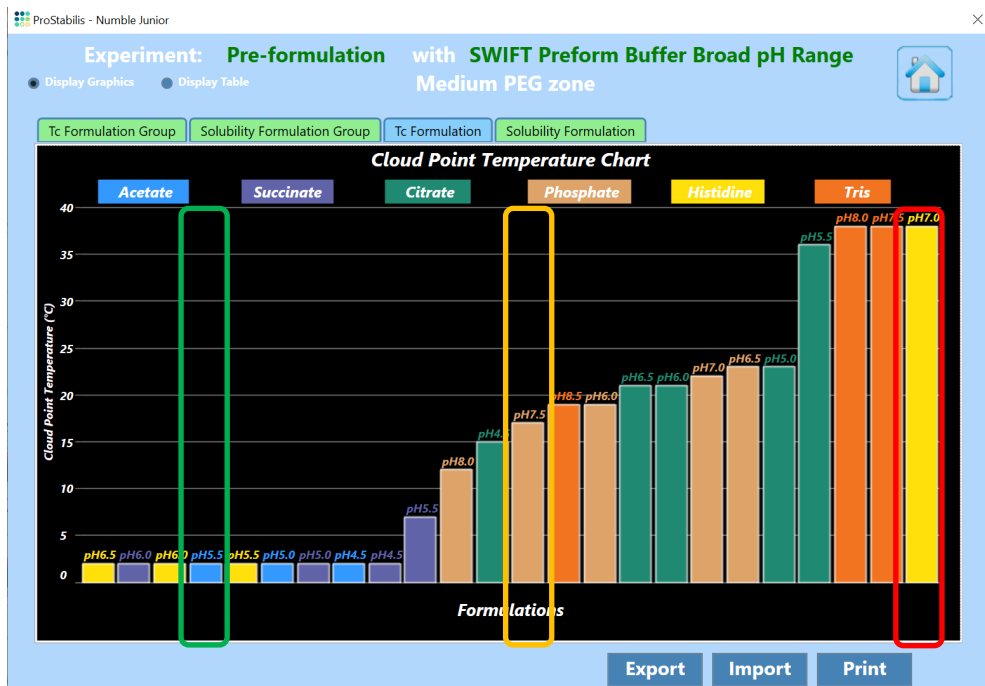


- Nimble Junior™ detects that this mAb has higher solubility at low pH.
- At pH 6.0, this mAb is more soluble in succinate and histidine than in phosphate and citrate.



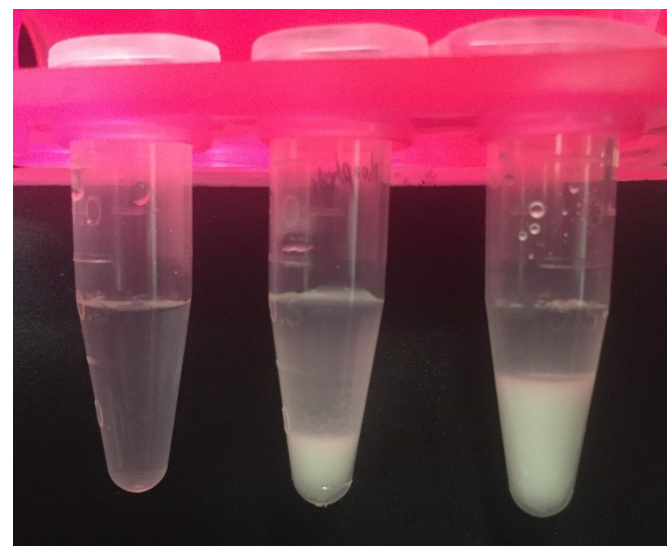
# Case II: Buffer/pH Screening for a mAb

Nimble Junior™ results agree with the direct solubility test by ultrafiltration



3 representative formulations with **high**, **medium**, and **low** solubility of this mAb detected by Nimble Junior

After up-concentration by ultrafiltration



acetate pH 5.5      phosphate pH 7.5      histidine pH 7.0

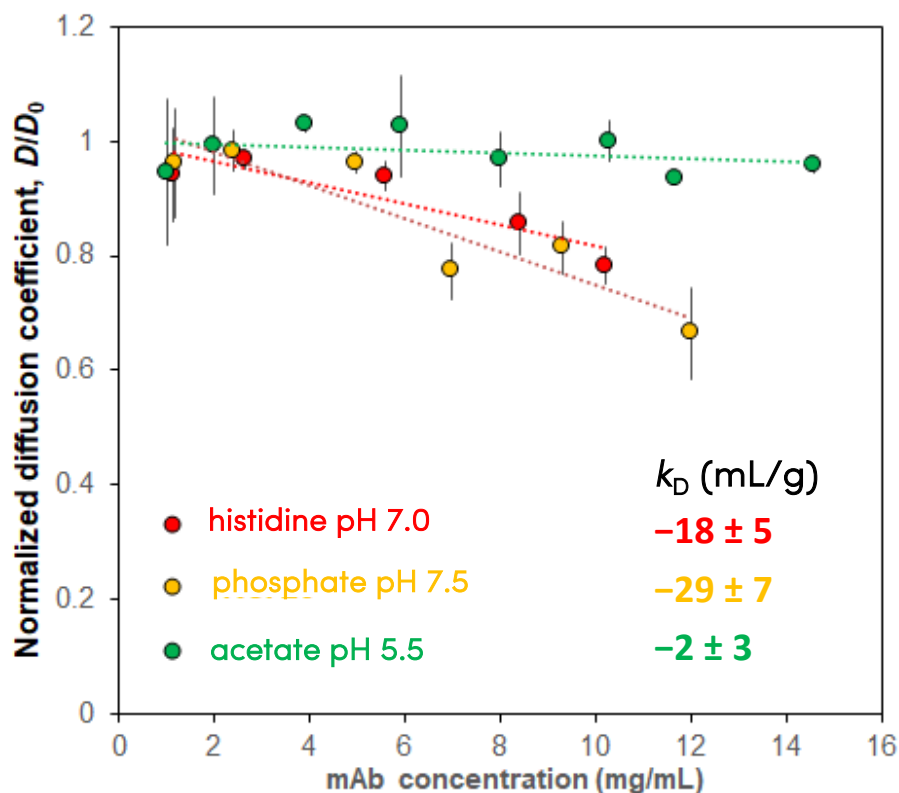
supernatant concentration

65 mg/mL      38 mg/mL      12 mg/mL

# Case II: Buffer/pH Screening for a mAb

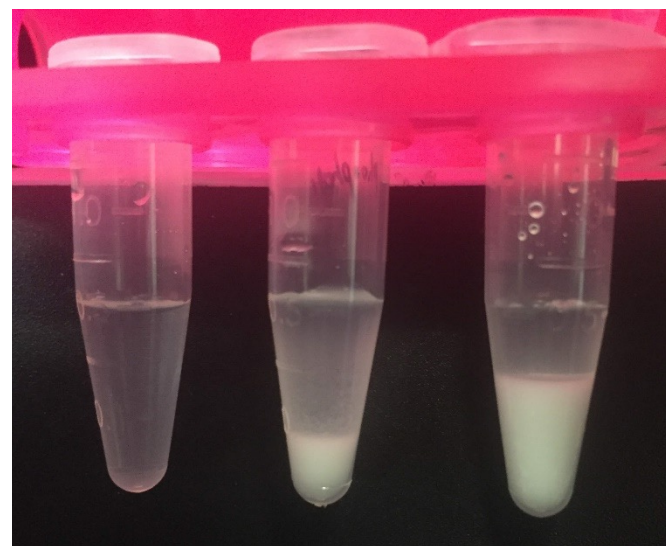
$k_D$  measurement for this mAb only differentiates acetate buffer; phosphate and histidine buffers are within the experimental error

## $k_D$ measurement by DLS



More negative  $k_D \Rightarrow$  stronger attraction

## Up-concentration by ultrafiltration



acetate pH 5.5      phosphate pH 7.5      histidine pH 7.0

supernatant concentration

65 mg/mL

38 mg/mL

12 mg/mL

# Specifications

Item	Specification
Sample temperature	0 °C – +90 °C
Light source	490 nm
Power	95 – 260 V, 50 – 60 Hz, 10 A
Dimensions	321 W x 397 D x 336 H (mm)
Weight	12 kg



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