Regeneration

Things to consider + Sensor Regeneration condition overview

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Regeneration

Regeneration is the process of restoring the sensor to its initial condition by removing the attached analyte without damaging the ligand on the sensor surface.

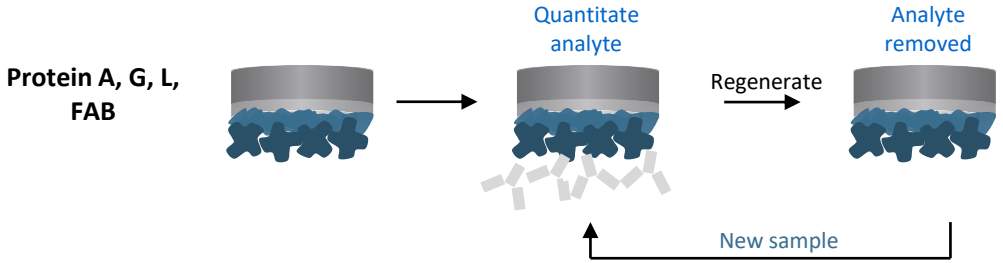
Regeneration of Current Biosensor Products

- This presentation will show:
  - **Regeneration conditions for current biosensor products.**
  - How to determine the right custom regeneration conditions.
  - How to use regeneration in the software.

Regeneration of Current Biosensor Products

- Biosensor regeneration is dependent on:
  - Capture chemistry and affinities
  - Assay requirements
- Regeneration of biosensors for quantitation applications must be more complete than for kinetics assays. This is because the quantitation results are more dependent on surface capacity of the sensor.
  - For example, a loss of 20% capacity over 10 regeneration cycles will not affect kinetic constants, but would affect precision of quantitation by 10-20%.

Protein A/G/L and FAB2G Biosensors



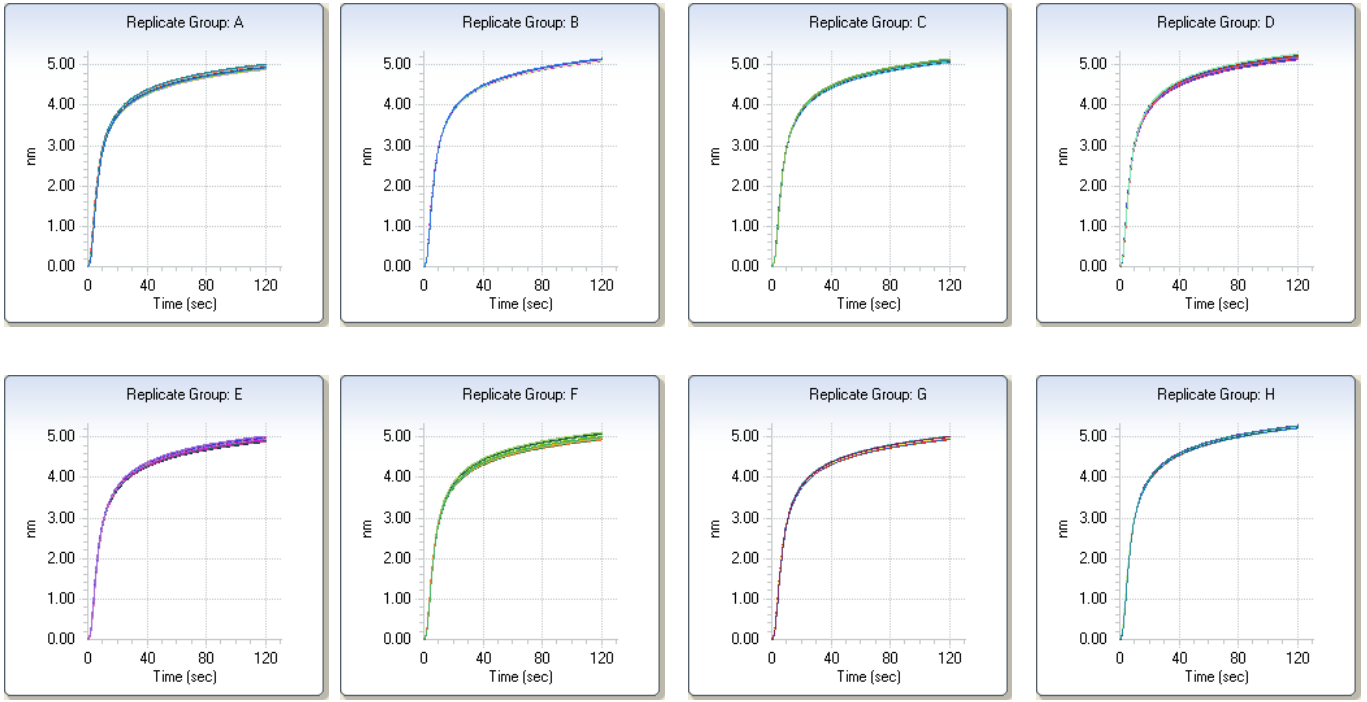
- Optimal Regeneration Buffer

ProA	10mM Glycine pH 1.0-1.5
ProG	10mM Glycine pH 1.7-2.0
ProL	10mM Glycine pH 1.5
FAB	10mM Glycine pH 1.7

- Be sure to use the pre-conditioning option for best precision
- 3 cycles of 5-10 seconds alternating with running buffer as a neutralizations step

Regeneration of ProA binding 500 ug/mL hIgG

Data shown by sensor. Each graph shows binding curves from 10 binding cycles (9 regenerations)



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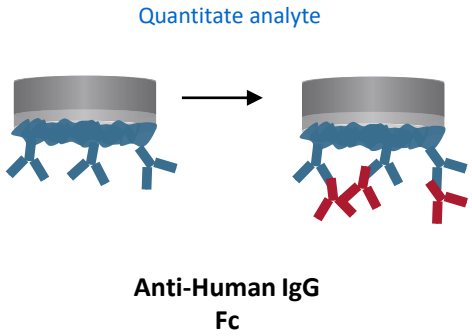
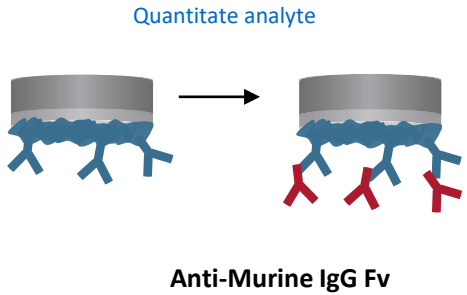
Regeneration efficiency of Protein A Biosensors

	Ave. calculated concentration of 8 channels (ug/mL)	%CV of 8 channels
REG0	485.0	5%
REG1	485.2	4%
REG2	496.1	5%
REG3	490.0	5%
REG4	490.5	5%
REG5	503.4	5%
REG6	505.1	5%
REG7	500.7	4%
REG8	501.4	4%
REG9	498.9	4%
CV over 9 regenerations		**2%**

Regeneration protocol:
3 Cycles of 10 mM Glycine pH 1 for 5 sec at 200 rpm, Sample Diluent for 5 sec at 200 rpm

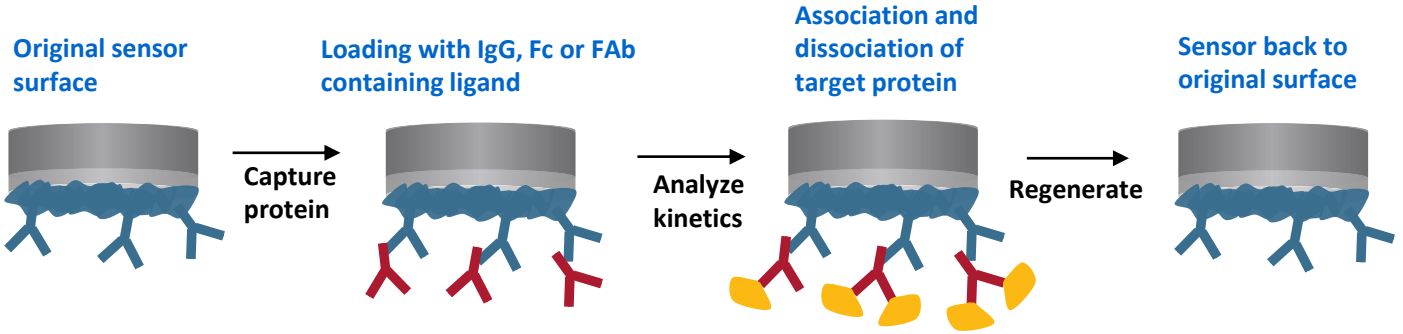
AHQ and AMQ Biosensor





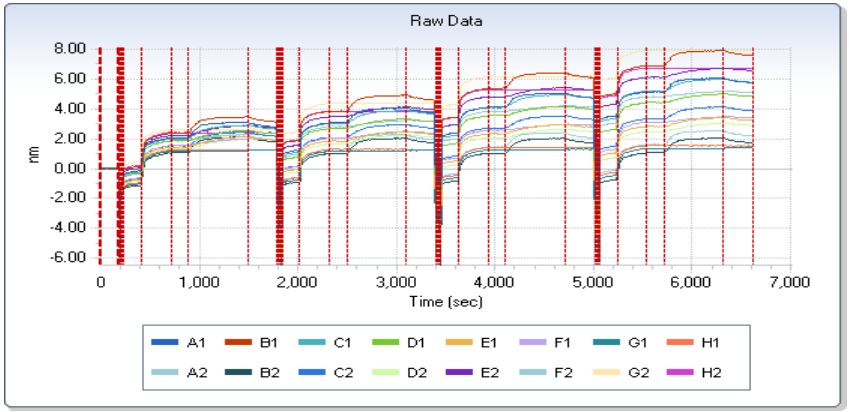
- Regeneration not recommended due to:
  - High affinity of capture antibody. Requires harsh conditions that damage the surface.
  - Pfizer SSF is regenerating this sensor, but they are using it only for kinetic epitope binning assays since loss of surface capacity is evident.

AHQ and AMQ Biosensor



- Optimal Regeneration Conditions:
  - 10mM Glycine pH = 1.7 (3 cycles of 5 seconds alternating with running buffer as a neutralizations step)
  - Pre-conditioning of the sensor surface is required for accurate kinetics.

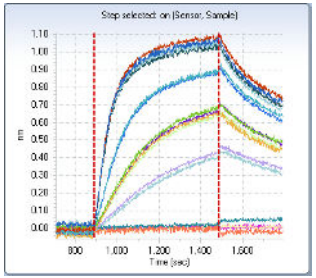
Kinetics analysis using AHC Biosensors with regeneration



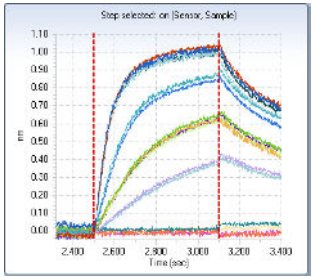
Full assay

KD (M)	Kon (1/Ms)	Kdis (1/s)
3.66E-09	3.97E+05	1.45E-03
3.45E-09	4.06E+05	1.40E-03
3.75E-09	3.89E+05	1.46E-03
3.94E-09	3.84E+05	1.51E-03
3.99E-09	3.77E+05	1.50E-03

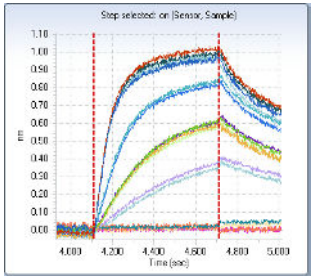
Cycle 1



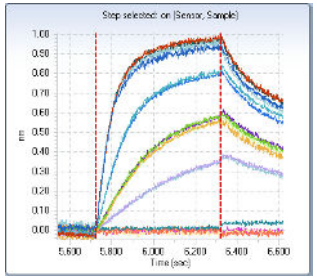
Cycle 2



Cycle 3



Cycle 4



Note 10% loss after 3 cycles, yet kinetic constants stay the same

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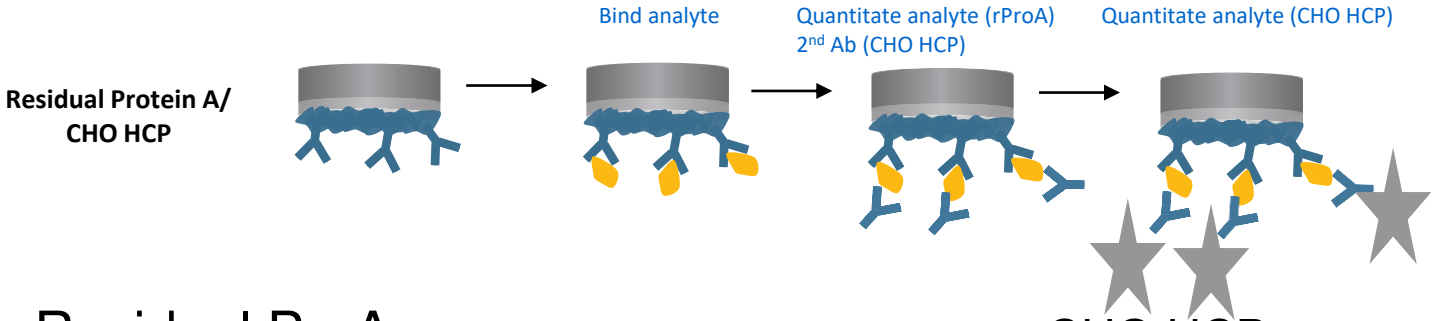
Residual ProA/CHO HCP kits



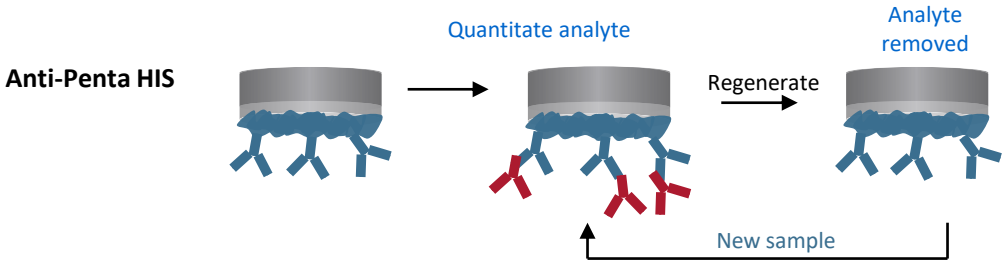
Residual ProA

- Regeneration not recommended due to:
  - High affinity of capture antibody. Requires harsh conditions that damage the surface.
  - Kit contains only enough reagents to support use of each biosensor once.

CHO HCP

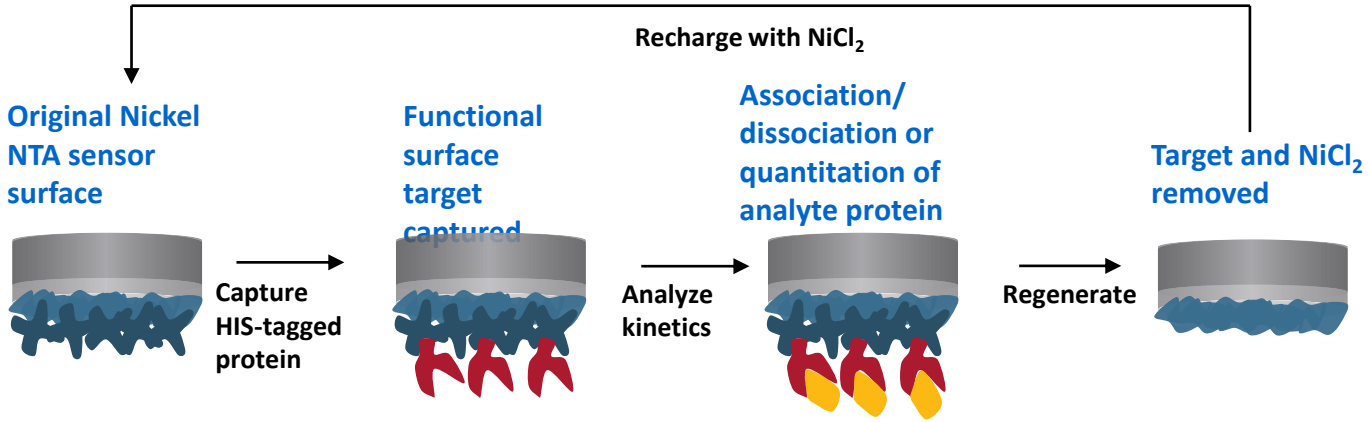
- Regeneration not recommended due to:
  - Kit contains only enough reagents to support use of each biosensor ones.
  - DAB precipitates on sensor surface and is not regenerable

HIS1K and HIS2 (Anti-PentaHIS) Biosensors



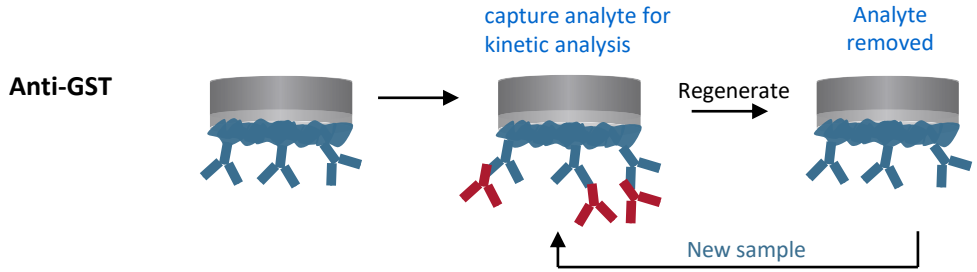
- Regeneration possible if:
  - Affinity for analyte is sufficiently low allowing full clearing of the surface without loss of surface capacity.
  - Generally this is not possible for quantitation, however Centocor is regenerating for a kinetic characterization assay.

Ni-NTA Biosensor



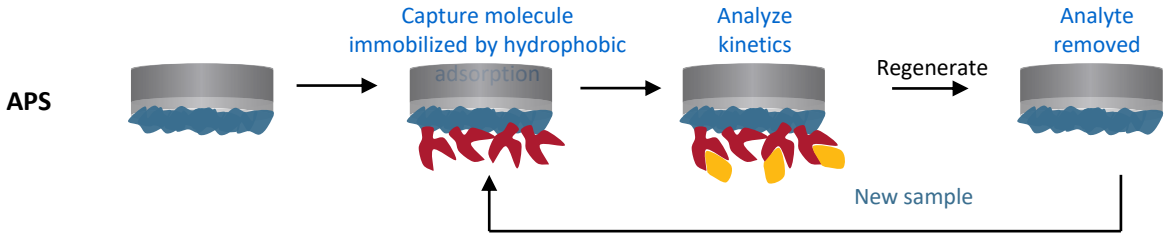
- Optimal Regeneration Conditions:
  - 10mM Glycine pH 1.7 - 3 cycles of 5-10 seconds alternating with running buffer as a neutralizations step
  - After the final cycle, sensor must be recharged with 10mM NiCl2 in water
  - DO NOT use preconditioning
  - DO NOT regenerate for Q applications (surface is totally different after each recharge)

Anti-GST Biosensor



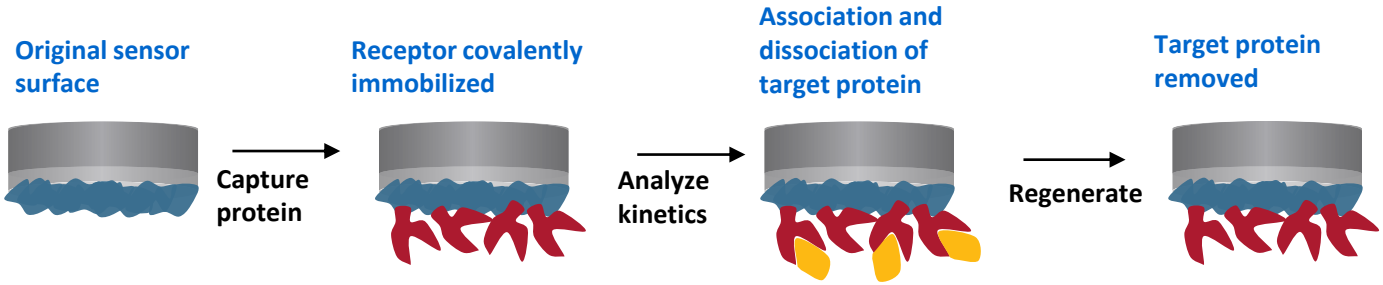
- Optimal Regeneration Buffer for kinetic applications
  - 10mM Glycine pH 1.7-2.0
  - DO NOT use pre-conditioning for best precision.
  - 3 cycles of 5-10 seconds alternating with running buffer as a neutralization step.
  - Note that there will be 2-3% surface capacity loss with each regen cycle (do not regenerate for quantitative applications).

APS Biosensor



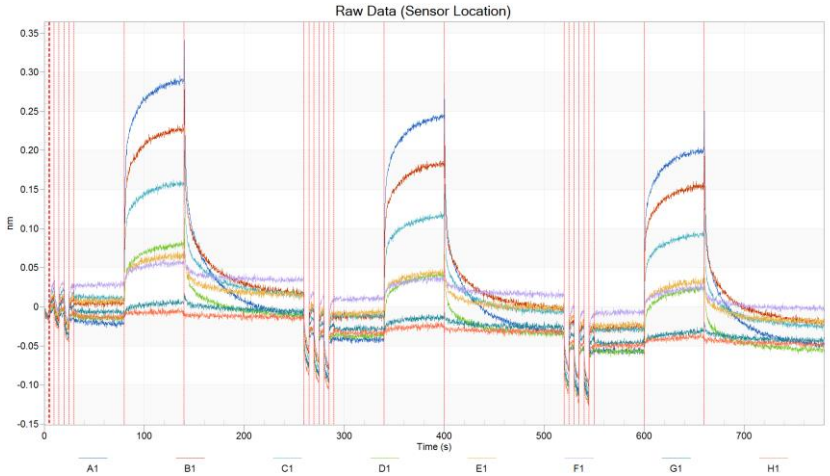
- Optimal Regeneration Conditions:
  - Are extremely variable and depend totally on what is attached.
  - Generally detergent solutions work well with this biosensor (1% Triton X-100, Tween-20, SDS etc).
  - If using a detergent it is very important to properly wash the biosensor clean before the next binding step.

AR2G Biosensor



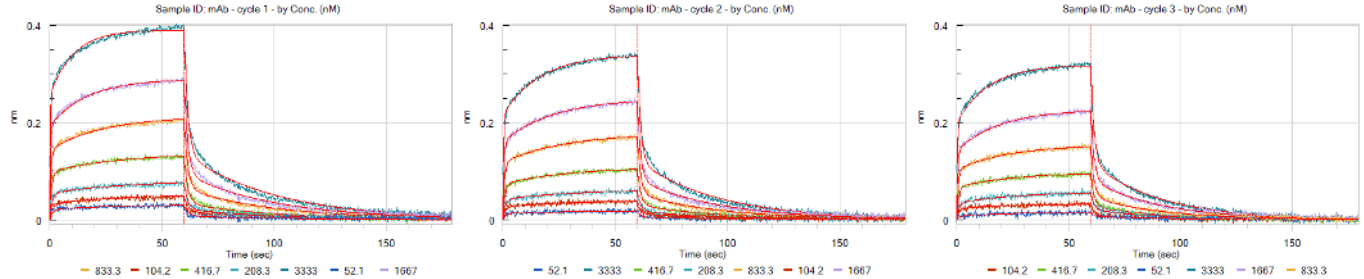
- Optimal Regeneration Conditions:
  - Are extremely variable and depend totally on what is attached.

Kinetics analysis using AR2G Biosensors with regeneration



Full assay

Sample ID	KD (M)	KD2 (M)
mAb - cycle 1	4.57E-07	1.15E-06
mAb - cycle 2	1.01E-06	2.82E-06
mAb - cycle 3	7.55E-07	2.83E-06



Note 25% loss after 3 cycles, yet kinetic constants stay the same

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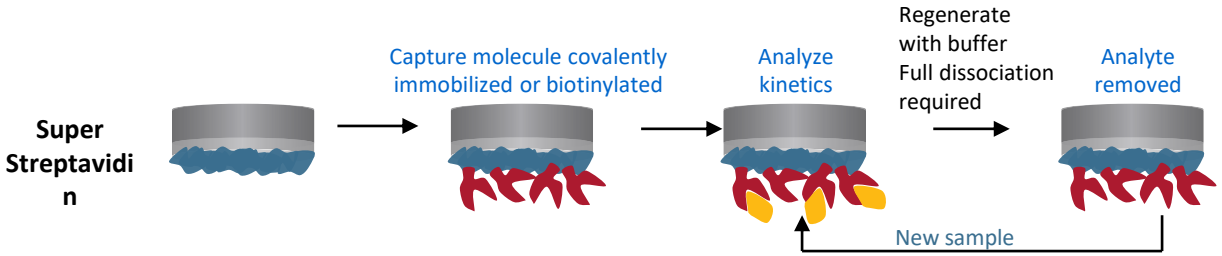
Chemical Stability of the AR2G Biosensor

Reagent	Maximum validated exposure time*
10 mM Acetate buffer pH 0.5, 1, 2, 3	15 minutes
10-100 mM Citrate buffer pH 2	15 minutes
KOH pH 9, 10, 11	15 minutes
50 mM NaOH	15 minutes
Phosphoric acid pH 2 + 5% Tween 20	15 minutes
SDS (0.5%, 0.1%, 0.05%)	5 minutes
5M NaCl	15 minutes
4M MgCl2	15 minutes
1mM HCl	15 minutes
Ethylene glycol (25%, 50%)	15 minutes
20 mM EDTA	5 minutes
Mixture of 0.46 M KSCN, 1.83M MgCl2, 0.92 M urea and 1.83M guanidin-HCl	5 minutes
CHAPS (1%, 0.3%, 0.05%)	Not recommended
Mixture of equal amount DMSO, formamide, ethanol, acetonitrile and 1-butanol	5 minutes

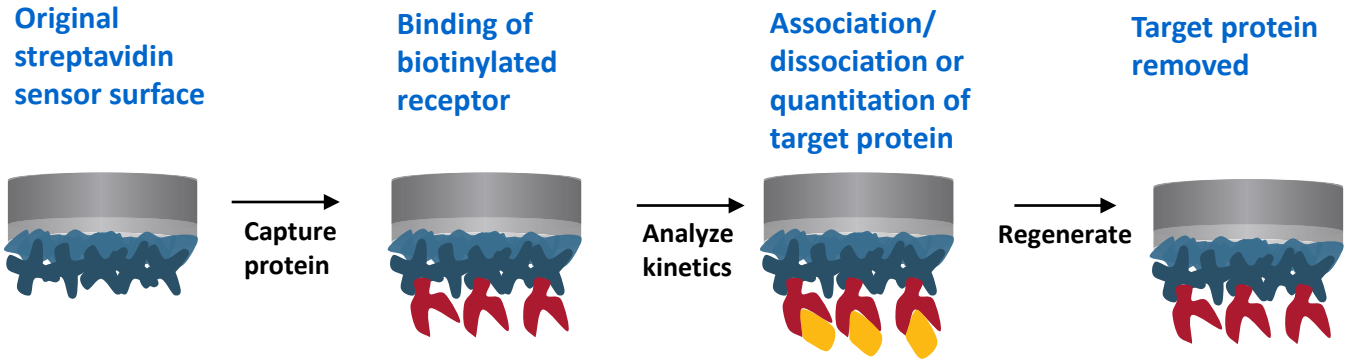
*** Exposure times are for the biosensor itself. Stability of the protein studied will be protein dependent.**

SSA (Super Streptavidin) biosensor



- Optimal Regeneration Conditions:
  - Neutral buffers only!
    - Small molecules of low affinity should dissociate fully in running buffer.
  - Chemical regeneration of this biosensor will damage the surface preventing proper use in small molecule analysis

SA/SAX/SAX2 Biosensor



- Optimal Regeneration Conditions:
  - Are extremely variable and depend totally on what is attached.
  - Note, if this biosensor is used in a multistep quantitation assay that utilizes precipitating substrate, it cannot be regenerated.

Chemical Stability of SA/SAX/SAX2 Biosensor

Reagent	Maximum validated exposure time*
HCl (pH 0.5, 1.0, 1.5)	15 minutes
NaOH (pH 10, 11)	15 minutes
NaOH (pH 12, 12.5, 13)	Not recommended
10 mM Glycine (pH 1, 2, 3)	15 minutes
NaCl (1, 2.5, 5 M)	15 minutes
MgCl2 (0.1, 0.5, 1 M)	15 minutes
Tween-20 (0.1%, 0.25%, 0.5%)	15 minutes
SDS (0.05%, 0.1%, 0.25%, 0.5%)	Not recommended
SDS (0.005%, 0.01%)	15 minutes
Phosphoric Acid (50, 100, 250, 500 mM)	15 minutes
EDTA (25, 50, 100 mM)	15 minutes
TritonX-100 (0.1%, 0.25%, 0.5%)	15 minutes

*** Exposure times are for the biosensor itself. Stability of the protein studied will be protein dependent.**

Regeneration of Current Biosensor Products

- Quick Overview:

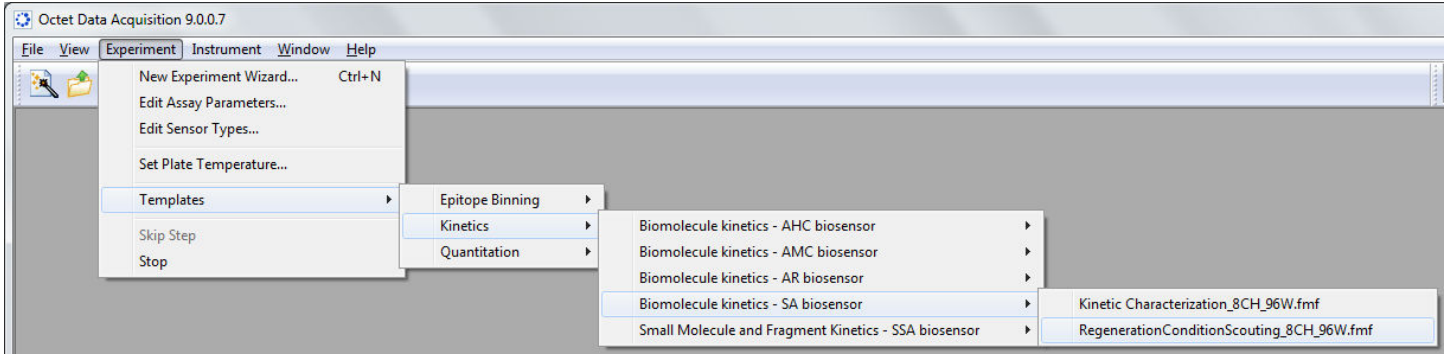
Antibody biosensors	Intended Application	Regeneration	Recommended buffer	Assay Parameters
ProA	Q	Yes	10mM Glycine pH 1.0-1.5	Precond, 3x 5-10s
ProG	Q	Yes	10mM Glycine pH 1.7-2.0	Precond, 3x 5-10s
ProL	Q	Yes	10mM Glycine pH 1.5	Precond, 3x 5-10s
FAB	Q/K	Yes	10mM Glycine pH 1.7	Precond, 3x 5-10s
AHC	K	Yes for K	10mM Glycine pH 1.7	Precond, 3x 5-10s
AMC	K	Yes for K	10mM Glycine pH 1.7	Precond, 3x 5-10s
AHQ	Q	No for Q	-	
AMQ	Q	No for Q	-	
Biosensor kits	**Intended Application**	**Regeneration**	**Recommended buffer**	**Assay Parameters**
rProA	Q	No for Q	-	
CHO HCP	Q	No for Q	-	
Anti-TAG biosensors	**Intended Application**	**Regeneration**	**Recommended buffer**	**Assay Parameters**
HIS1K	Q/K	Custom	Protein dependent	Protein dependent
HIS2	Q	Custom	Protein dependent	Protein dependent
NTA	Q/K	Yes for K, No for Q	10mM Glycine pH 1.7	3x 5-10s
FLG	Q/K	Yes for K, No for Q	10mM Glycine pH 1.7	Precond, 3x 5-10s
GST	Q/K	Yes for K, No for Q	10mM Glycine pH 1.7	3x 5-10s
Generic biosensors	**Intended Application**	**Regeneration**	**Recommended buffer**	**Assay Parameters**
APS	K	Custom	Protein dependent	Protein dependent
AR2G	K	Custom	Protein dependent	Protein dependent
SSA	K	Neutral pH only	Protein dependent	Protein dependent
SA	K	Custom	Protein dependent	Protein dependent
SAX(2)	Q/K	Custom	Protein dependent	Protein dependent

Regeneration of Current Biosensor Products

- This presentation will show:
  - Regeneration conditions for current biosensor products.
  - **How to determine the right custom regeneration conditions.**
  - How to use regeneration in the software.

Rapid Regeneration Scouting Acquisition Template

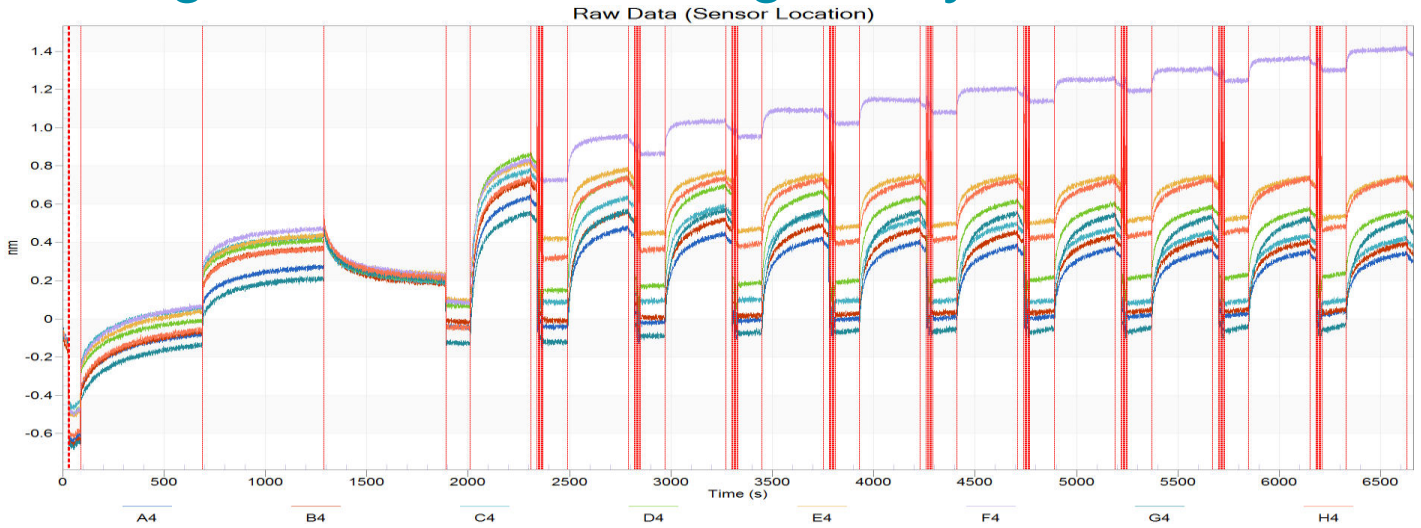
- Use of RegenerationConditionScouting Template embedded in the software:



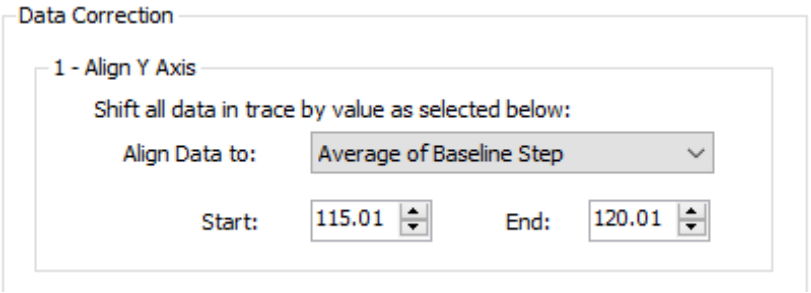
- Use 1 Ligand loading concentration and 1 high Analyte concentration and 8 different Regeneration Buffers.
- Copy Regeneration Buffer names in Sensor Info column on Sensor Assignment tab (3):

Well	Sensor Type	Lot Number	Information
A1	SA (Streptavidin)		5 M NaCl
B1	SA (Streptavidin)		0.01% SDS
C1	SA (Streptavidin)		NaOH, pH 10
D1	SA (Streptavidin)		NaOH, pH 11
E1	SA (Streptavidin)		HCl, pH 0.5
F1	SA (Streptavidin)		10mM Glycine, pH 1
G1	SA (Streptavidin)		10mM Glycine, pH 2
H1	SA (Streptavidin)		500mM phosphoric acid

Rapid Regeneration Scouting Analysis



- Analyze data both with and without step Align Y axis to baseline:



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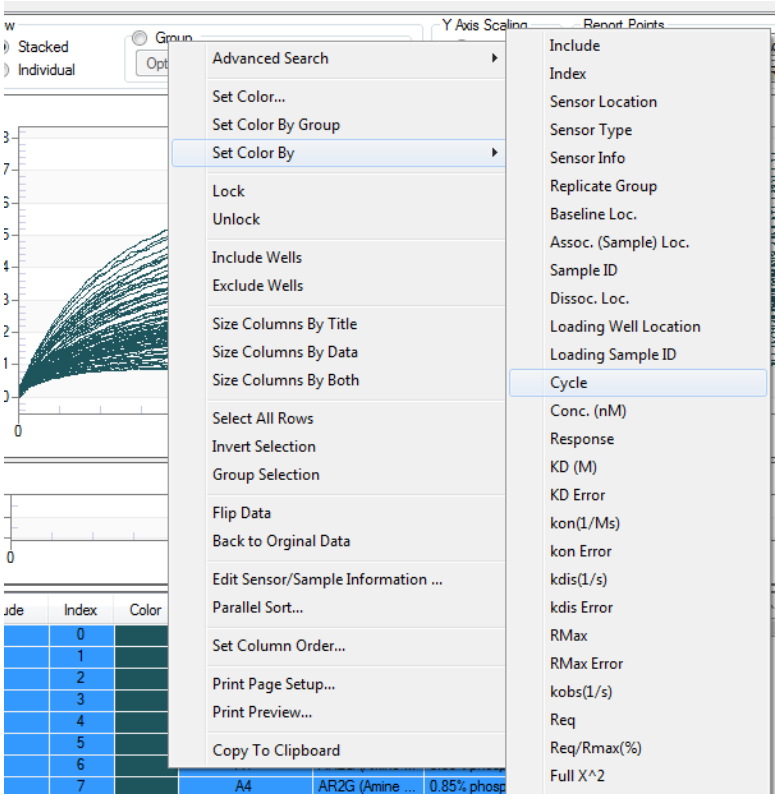
Rapid Regeneration Scouting Analysis

- Set Color by Cycle:



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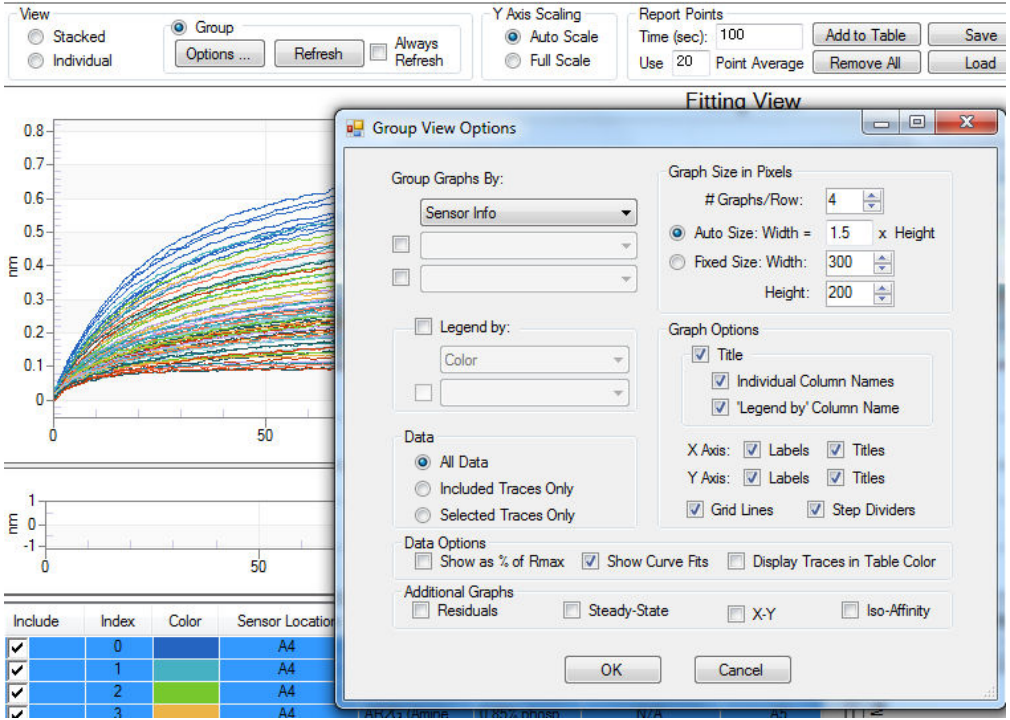
Rapid Regeneration Scouting Analysis

- Group data by Sensor Info:



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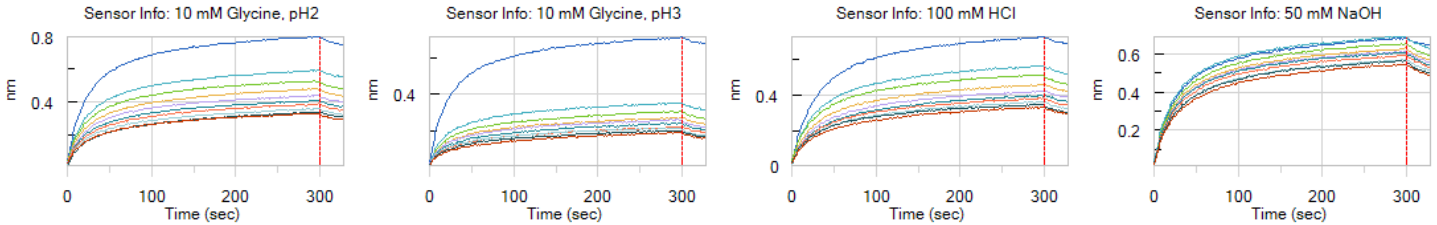
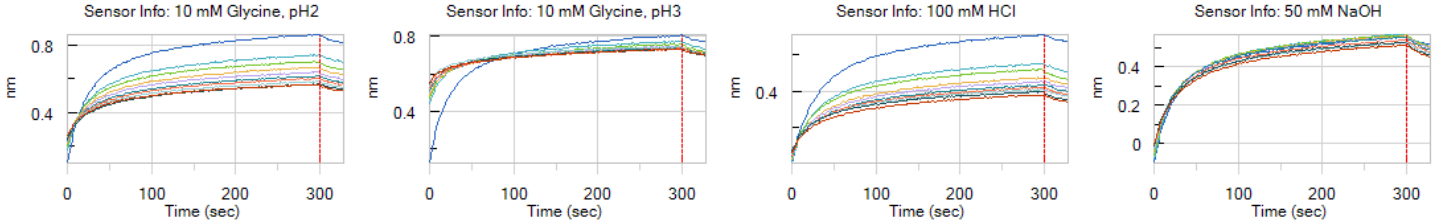
Rapid Regeneration Scouting Analysis



- When using Align Y axis, data shows overlay of association curves of different cycles and right regeneration buffer can be chosen.



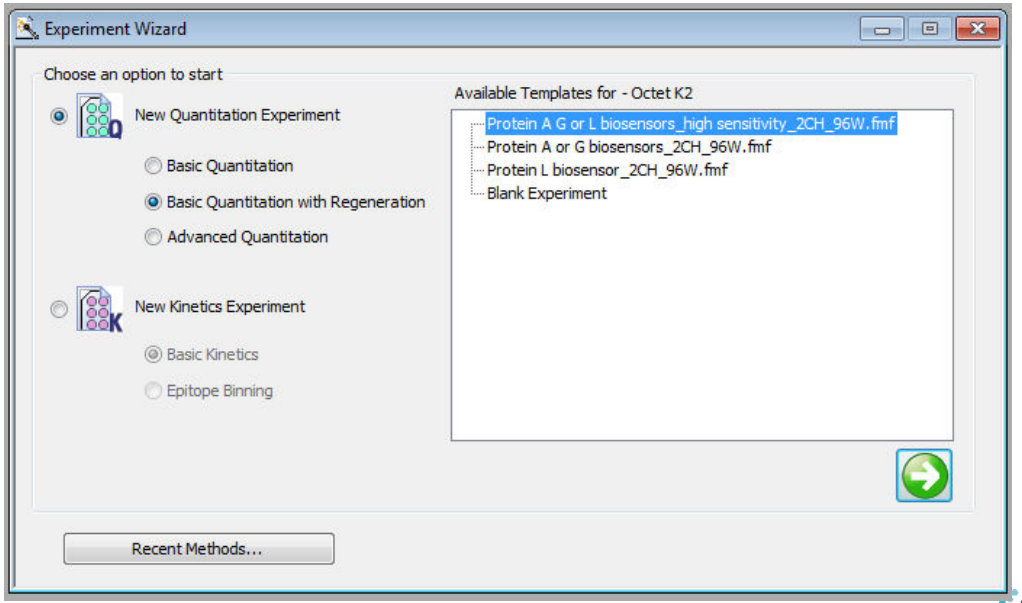
- When not using Align Y axis, data gives information if regeneration was too harsh for the ligand (starting point the same, association curves decrease in nm-shift) or if analyte did not come of sensor (starting point increases in nm-shift, association curves reach similar level in nm-shift).

Regeneration of Current Biosensor Products

- This presentation will show:
  - Regeneration conditions for current biosensor products.
  - How to determine the right custom regeneration conditions.
  - **How to use regeneration in the software.**

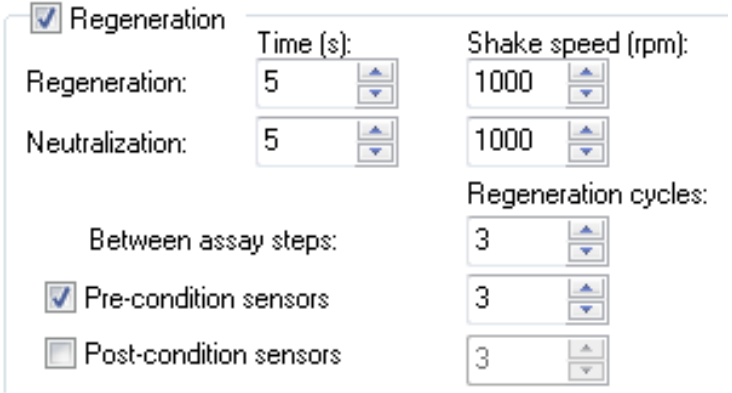
Regeneration in Quantification mode

- Regeneration possible in both
  - Basic Quantitation with Regeneration
  - Advanced Quantitation



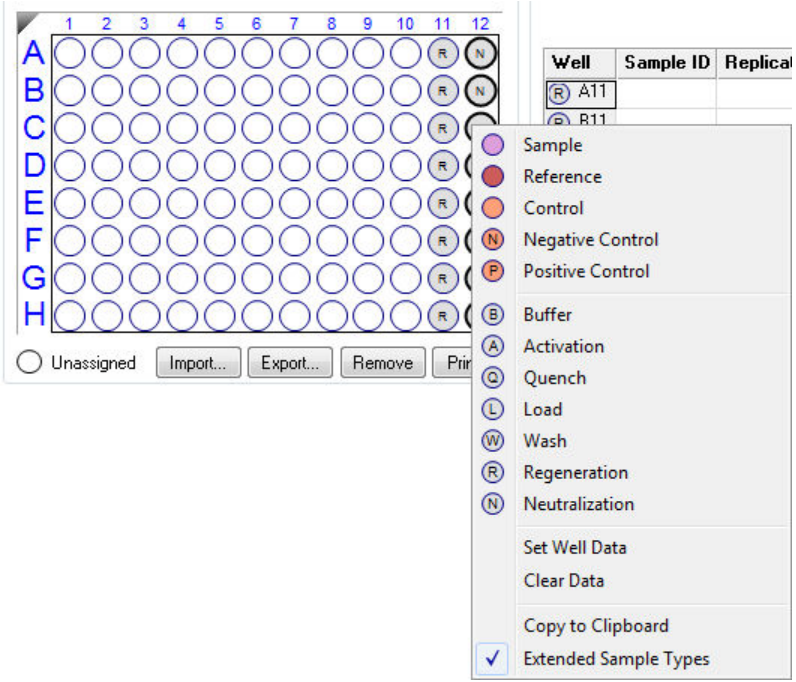
Regeneration in Quantification mode

- Standard Assay parameters for regeneration are:
  - 5 secs Regeneration at 200/1000 rpm
  - 5 secs Neutralization at 200/1000 rpm
  - 3 cycles of above steps
- Differentiation can be made between amount of cycles for pre-conditioning and between assays.



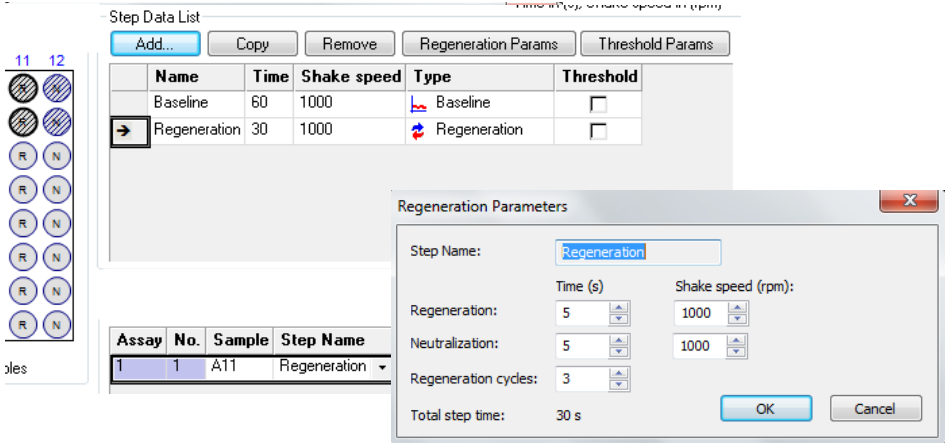
Regeneration in Kinetic mode

- Add Regeneration and Neutralization column to your plate



Regeneration in Kinetic mode

- Add Regeneration step to your assay step list (change parameters using Regeneration Params button)



- Double-click on Regeneration column to have Regeneration step added to the assay
- Note that this Regeneration Step defined by the software will put the nm-shift after last Neutralization step automatically at 0 nm.

Thank You

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