PerkinElmer®
For the Better

July, 2012

EnSpire

Multilabel Plate Reader

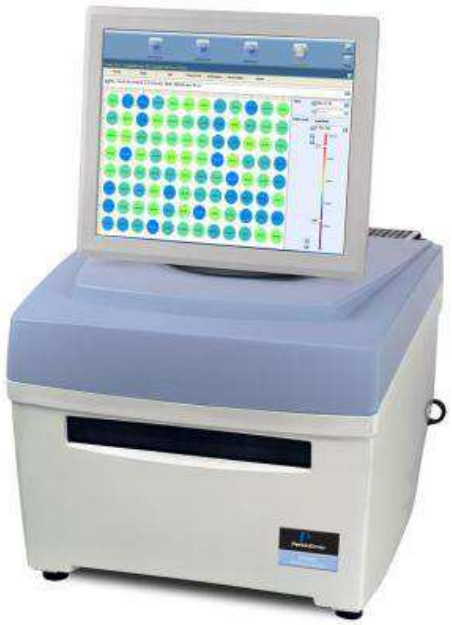
博克科技有限公司
產品應用專員 曾筱筑

EnSpire Modular design wih individual fixed optics

- Fluorescence Intensity
- UV/Absorbance

PKI's high performance
Quad monochromator technology

- Ultra-sensitive luminescence
- Alpha technology
- High-end Temperature control (up to 65℃)
- 2 Injectors with heating and Stirrer function
- Touch Screen
- Intuitive, user friendly software



EnSpire with Quad Monochromators

EnSpire – with Monochromator

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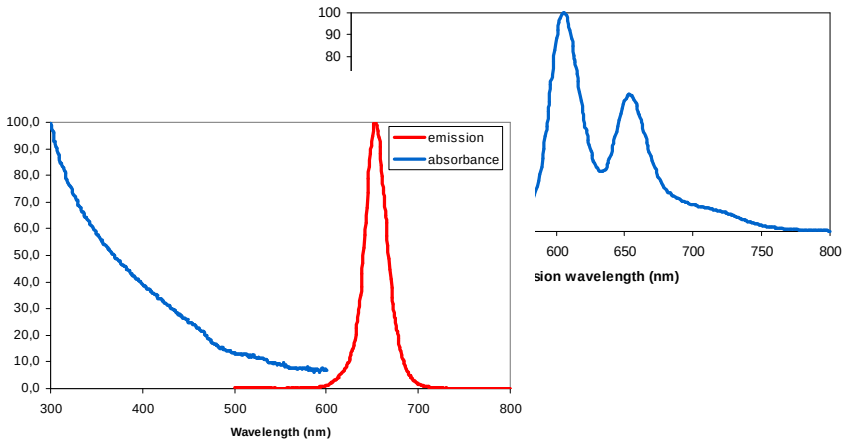
EnSpire (monochromator)

FOR QUANTITATION

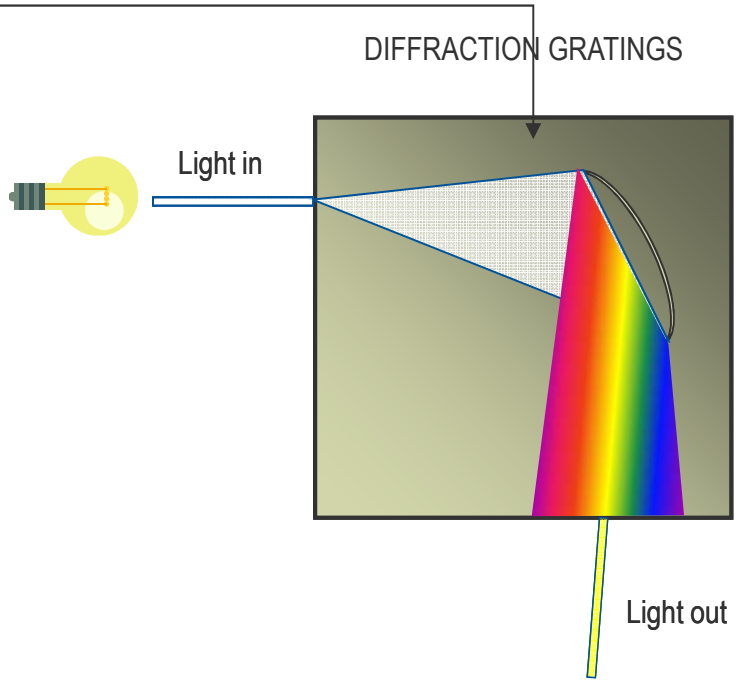
Absorbance & Fluorescence



EnSpire Monochromator Applications

1. Fluorescent assays
2. Absorbance assays
   - ELISA (absorbance)
   - Qantitation assays (UV/VIS absorbance)
     - Protein (A280)
     - Bradford (A405)
     - DNA/RNA (A260/A280)

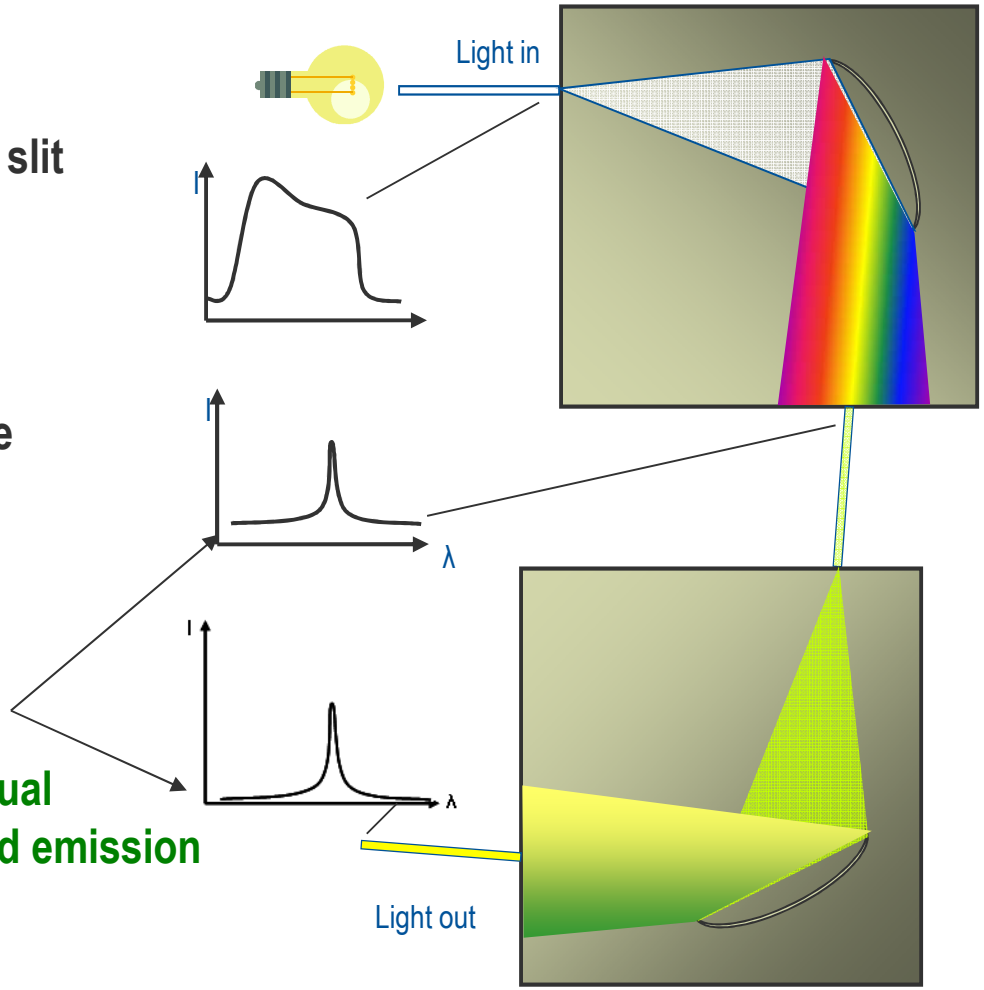
- Monochromators use diffraction gratings to physically separate the individual wavelengths present in the white light → easy wavelength selection and scanning



Benefits of Quad Monochromators

- The filtering capacity of single monochromator is limited
  - Unwanted wavelengths pass the slit
  - Increased background noise

- Dual monochromator filters light twice
  - Lower assay background noise
  - Higher S/B



A Quad monochromator system uses dual monochromators for both excitation and emission

Features and benefits – *Monochromators*

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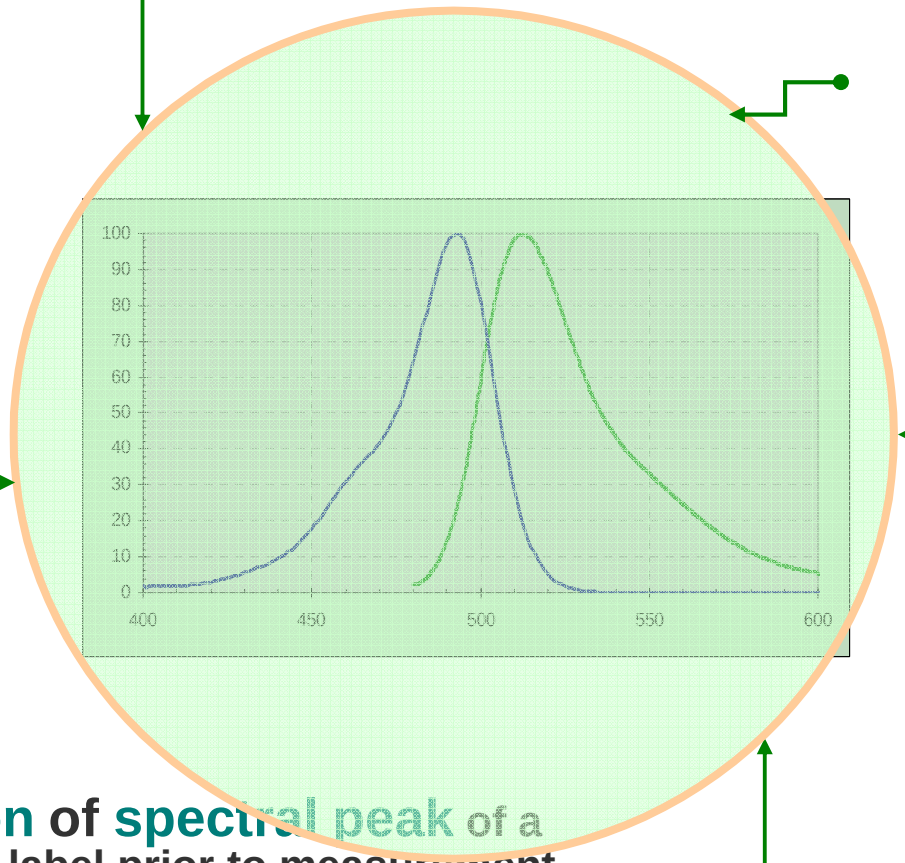
Narrow stokes shift labels
Applications: FRET YFP-GFP, SNP

Flexibility easy wavelength selection
Applications: ALL

Spectral scanning
Applications: ALL

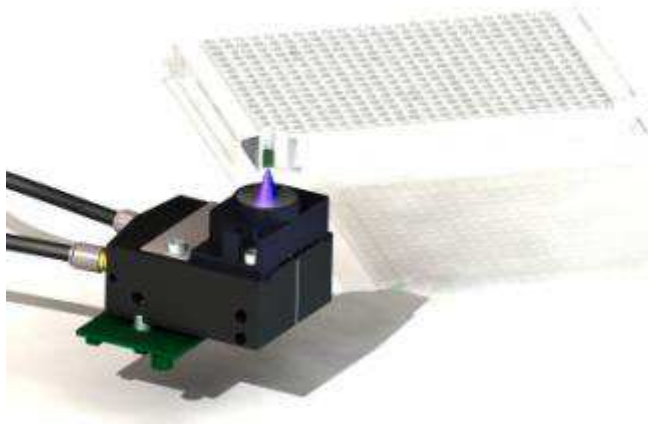


Problem solving
e.g. in case of no signal with filters
Applications: ALL

Verification of spectral peak of a fluorescence label prior to measurement with filters
Applications: ALL

EnSpire with Monochromator

- Wavelength is selectable from 220 nm to 850 nm.
- Wavelength selection for excitation & emission
- Tunable in 0.1 nm increment
- Bottom-reading



EnSpire with Monochromator

- The excitation is done from top and detection is done from bottom.
- Wavelength range: 230-1000 nm

EnSpire with Alpha technology

AlphaScreen and AlphaLISA technology

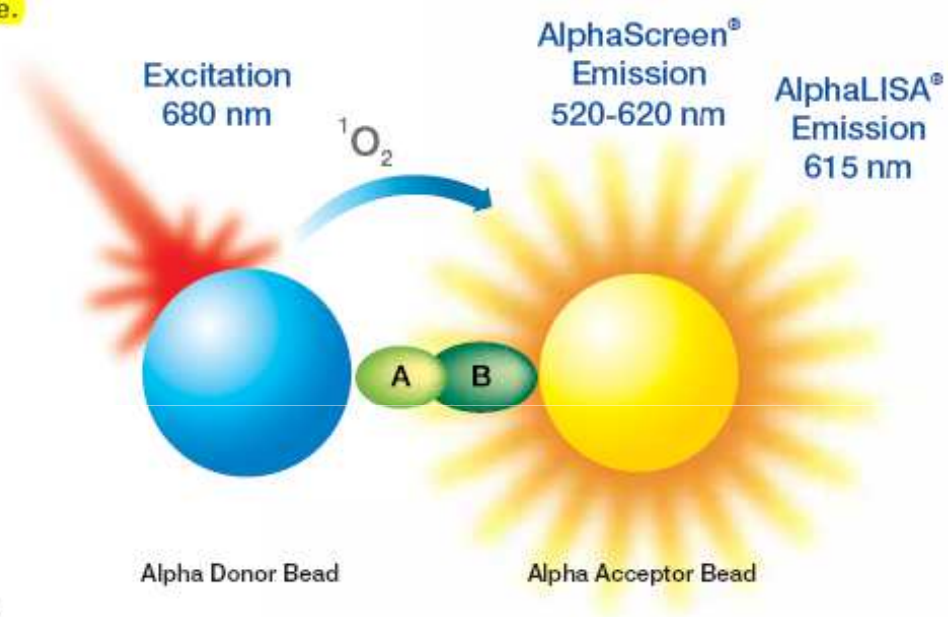
Alpha technology

Amplified luminescent proximity homogeneous assay

- Amplified → Signal intensity
- Luminescent → Reaction measurement
- Proximity → Distance between Alpha Donor and Acceptor beads
- Homogeneous → No wash necessary
- Mix and measure!

Alpha Technology

Illumination at a long wavelength ensures that few biological or assay substances will interfere.

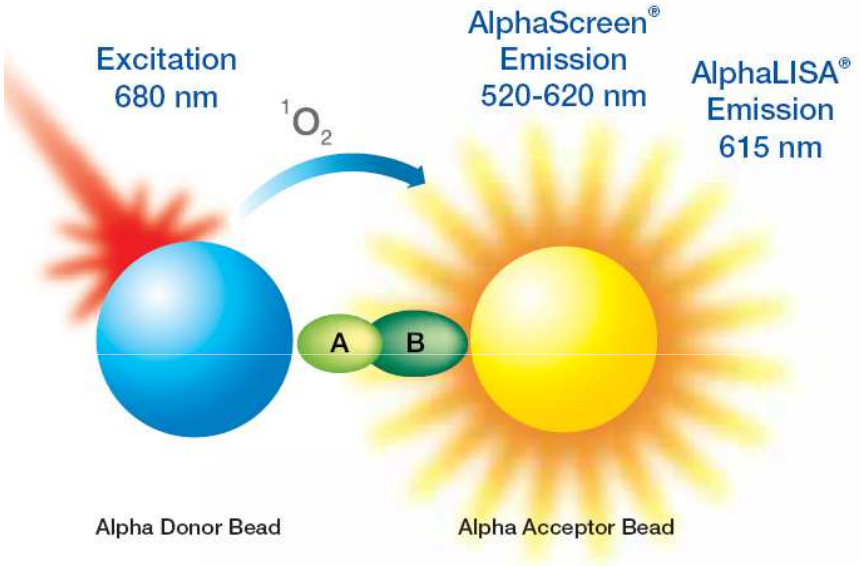


Singlet oxygen can travel up to 200 nm in solution, allowing the measurement of very large biological molecules.

A high concentrate of photosensitizer in each Alpha Donor bead generates up to 60,000 singlet oxygen molecules per second. This results in a very high signal amplification that contributes to detection sensitivity to the attomole level.

The Acceptor beads contain a thioxene derivative that reacts with the singlet oxygen molecule to generate a chemiluminescence reaction. This energy is transferred to fluorophores within the same bead, shifting the emission to 520-620 nm in the case of the AlphaScreen beads and 615 nm with AlphaLISA beads. A half-life decay reaction of 0.3 sec allows detection in a time-resolved mode.

Alpha Technology



- A homogeneous, bead-based proximity platform
- Detects virtually any molecule from large endogenous protein complexes to very small peptides
- Works with a variety of sample types including, serum, plasma, cell lysates, cell supernatant and purified reagents
- NO wash steps are required, improving workflow and making it easily automated
- Available in a variety of detection kits for pre-validated assays with off-the-shelf reagents

all in one well and with no wash steps

Steps of ELISA vs. AlphaLISA

ELISA*

Add assay buffer, matrix solution, standard (or sample)

↓

Incubate 1.5 hour on orbital shaker

↓

wash wash wash

↓

Add antibody detection solution

↓

Incubate 1.5 hour on orbital shaker

↓

Remove solution

↓

wash wash wash

↓

Add enzyme

↓

Incubate 30 minutes on orbital shaker

↓

Remove solution

↓

Add substrate

↓

Incubate 30 minutes on orbital shaker

↓

Read

AlphaLISA**

Add assay buffer, matrix solution, standard (or sample) ;
Add biotin antibody and AlphaLISA acceptor beads

↓

Incubate 30 – 60 minutes (RT)

↓

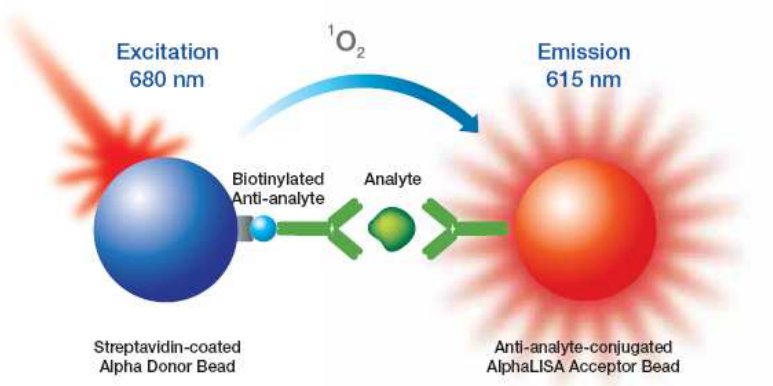
Add donor beads

↓

Incubate 30 – 60 minutes (RT)

↓

Read



AlphaLISA Benefits

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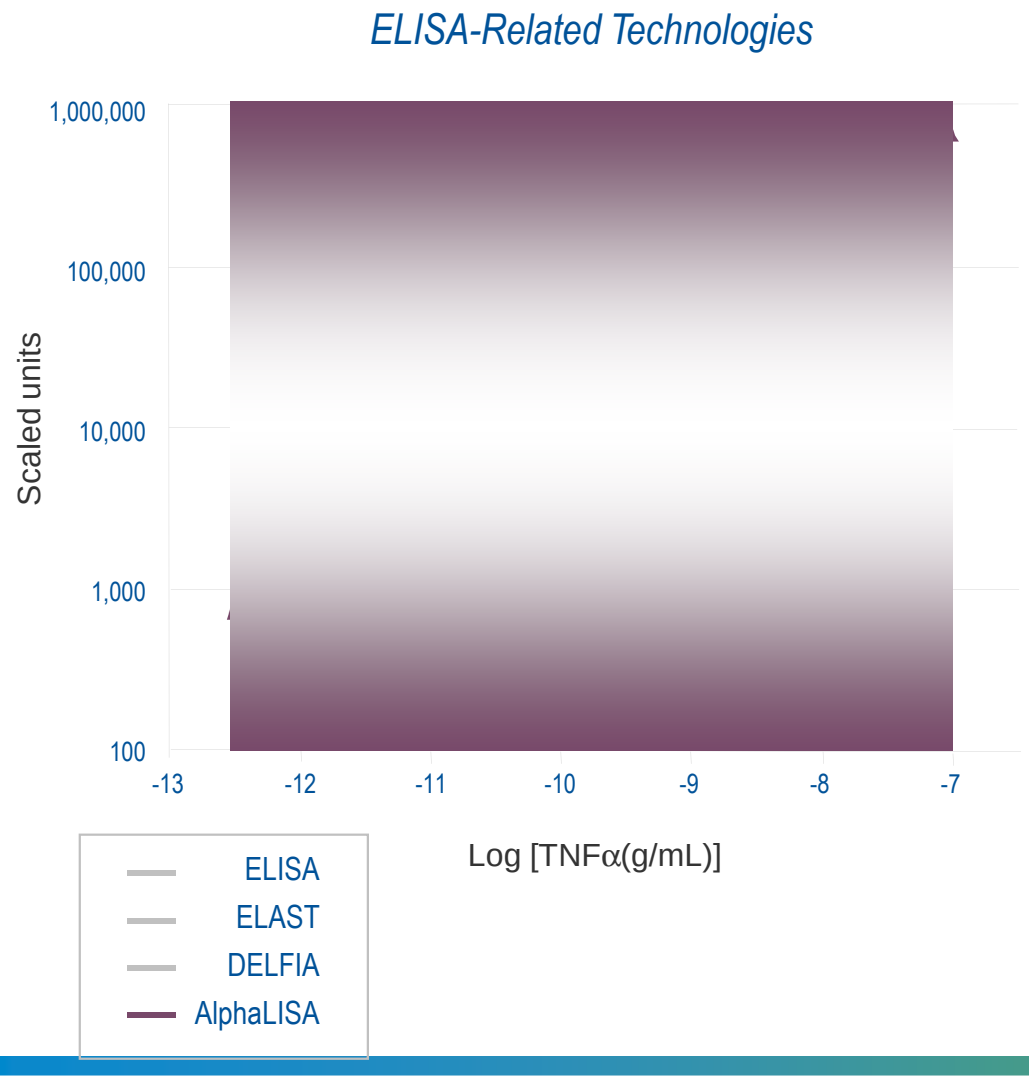


Highly sensitive

- Save money
- Reduce need for large volume of samples

Wide dynamic range

- Decrease dilutions

Homogeneous technology

- Time for work (increased productivity)
- Time to publish
- Decrease sample prep (improve work flow)

Proximity based

- Detection of simple to large complex biological interactions
- Suited for analytes from various sources including serum and plasma

Validated on PerkinElmer Instruments

Note: TNFα detection with a standard ELISA kit and alternative methods

EnSpire Alpha technology

- The Alpha technology uses laser for excitation and photomultiplier tube (PMT) for detection.
- 680 nm excitation with semiconductor laser
- The photomultiplier tube is located as close as the well as possible in order to maximize the sensitivity

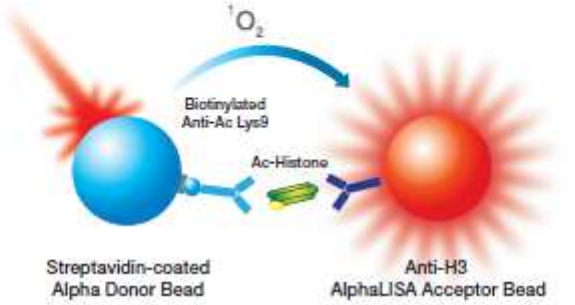
EnSpire Alpha technology

- AlphaScreen measurements are done in a “fly” mode,
  - Excitation is done for one well while the emission is done for another, during this the plate is constantly moving

- Excitation and emission collection times are adjustable in the software.

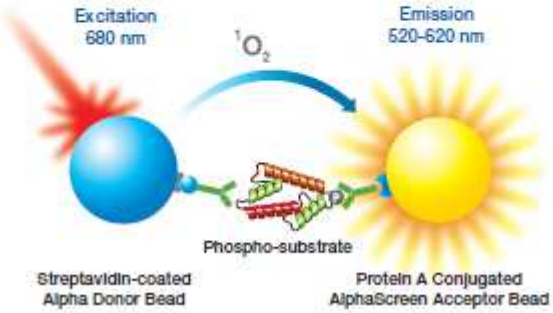
Receptor-Ligand

Acetyl-Histone Detection Assay



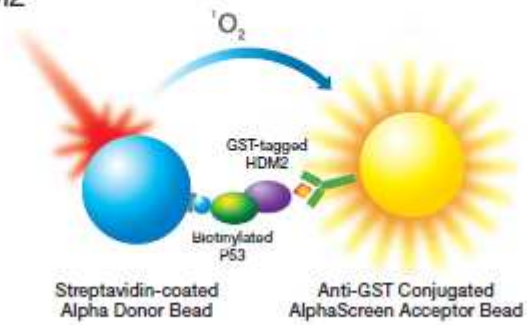
Kinase

EGFR



Protein:Protein

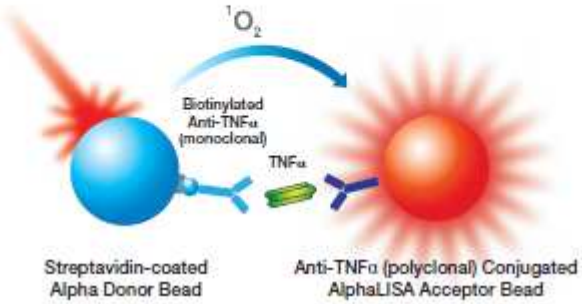
p53/HDM2



applications

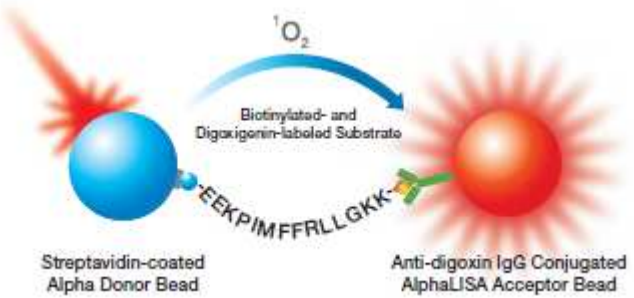
Immunoassay

TNFα detection – Sandwich format



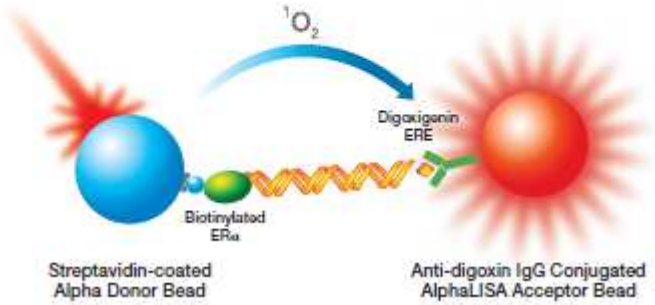
Protease

Cathepsin D – Cleavage of the specific substrate by cathepsin D results in a signal decrease



Protein-DNA Interaction

ERE-ERα interaction – Biotinylated protein



AlphaLISA® Immunoassay Kits

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Biologics

IgA
IgG
IgM
CHO-P
NSO-P
E.Coli HCP
Residual Protein A
IgE
SerumAlbumin

Angiogenesis

TNFa
VEGF
VEGFB
VEGFC
VEGFD

TNFa (mouse)

Cancer

AFP
CA125
CXCL11/I-TAC
EGF-R
EPO
ERBB2 / HER2
IFN-b
MMP1
MMP2
MMP9
b-NGF
PSA
TIMP1
TFF3
HGFR/c-MET
MMP3

Cardio-vascular

Myeloperoxidase
NT-proBNP
PCSK9
Plasminogen
Renin/Prorenin
tPA
a-2 macroglobulin
ICAM-1

Inflammation

CCL2 / MCP1
CCL3/MIP-1a
CCL4/MIP-1b
COMP
CRP
CXCL10/IP-10
G-CSF
GM-CSF
IFN-a
IFN-g
IL1α
IL1ß
IL2
IL3
IL4
IL5
IL6
IL7
IL8
IL10
IL12 (p70)
IL13
IL17
IL18
CXCL1/GRO-a
CXCL9/MIG
IL15

Inflammation

IFN-g (mouse)
IL1b (mouse)
IL6 (mouse)
IL10 (mouse)
IL2 (mouse)
IL7 (mouse)
IL15 (mouse)
IL17A (mouse/rat)
CCL2/MCP-1 (mouse/rat)

Neuro-degeneration

Aß 1-40
Aß 1-42
sAPPα
sAPPβ

sAPPa (C-term spec.)
sAPPb (high sensitivity)
Aß 1-15/16
Aß 1-40 (high spec.)
Aß 1-42 (high spec.)
Tau

Aß 1-40 (mouse/rat)
Aß 1-42 (mouse/rat)

Metabolic

Adiponectin
GH
GLP-1
IGF1
IGF2
Insulin
Leptin
Prolactin

Albumin (mouse)
C-peptide (mouse/rat)

Virology

HIV p24

AlphaScreen® Kits

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Immunity

Caspase 9
IkB
IKKalpha
IKKbeta
JNK (2)
Total JNK
NFkB p65
Stat-1
Stat-3
Stat-5
mTOR (2)

Internal Standard

Total GAPDH

Cancer

4EBP1 (2)
Akt (5)
Total Akt (2)
ALK (2)
Total ALK
ATF1
Caspase 9
Chk1
Chk2
EGF receptor
eiF4E
ELK-1
ErbB2
ERK
Total ERK
Histone H3
IkB
IKKalpha
IKKbeta
JNK (2)
Total JNK
c-Jun (2)
MEK-1
p53
Total p53
p70S6K (3)
PDK-1
RPS6 (2)
SMAD1
SMAD2
SMAD3
Stat-1
Stat-3
Stat-5
mTOR (2)
VEGF2 receptor

Diabetes

IGF-1 receptor
Insulin receptor

Inflammation

p38 MAPK
IkBalpha
IKKbeta
JNK (2)
Total JNK
c-Jun (2)
MKK3/6
MKK4
NFkB p65
SMAD1
SMAD2
SMAD3

Neuro-degeneration

CREB
BAD (2)
GSK3alpha
GSK3beta

Pathway kits

NFkB:
IKKalpha
IKKbeta
IkBalpha
NFkB
p38 MAPK

Akt:
Akt 308 (pan)
Akt473 (pan)
GSK3beta
p70S6K (T389)
Total Akt (pan)

MAPK:
MEK-1
ERK
p70S6K (T421/S424)
Elk1
Total ERK

EnSpire ultra-sensitive luminescence

EnSpire ultra-sensitive luminescence

- Dedicated PMT for reading luminescence
- PMT is positioned as close as possible over the well to read luminescence signal.

Comparing standardd to Ultra Sensitive Luminescence

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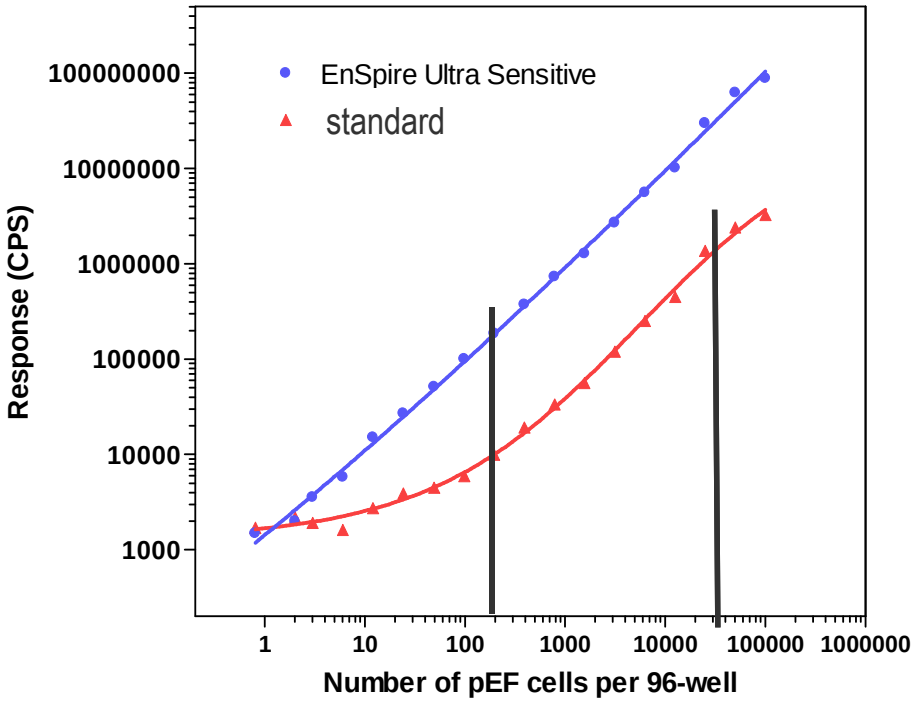
- Performance comparison between Standard and Ultra-sensitive luminescence
- Luminescence assay
  - ATPlite cell viability assay
  - Cell numbers ranging from 1 to 100000 cells per well

- Results
  - Ultra-sensitive luminescence shows 40 x lower detection limit
  - Measurement is linear over whole range of the assay in ultra-sensitive mode whereas it shows only limited linearity is standard mode



EnSpire with dispenser

EnVision unique Dispenser Unit

Dispenser Unit:

- ✓ **2 syringes**
- ✓ **Waste pump**
- ✓ **Liquid Temperature control system**
- ✓ **Magnetic Stirrer**

- ✓ The dispenser unit is equipped with two pumps
- ✓ Integrated hot plate (from ambient + 4 °C to 65 °C) and magnetic stirrer (100 - 500 rpm)
- ✓ Dispensing up to 384-well plates
- ✓ Volumes between 1µL .. 475µL
- ✓ **Dispense increments 0.5 µl steps**

EnSpire Software

Navigation panel – Your Main Dashboard

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- **Always on top**
- **4 Main Functions to Operate**
  1. Run Assay
  2. Create Protocol
  3. Edit Protocol
  4. Show Results

Push button to select meny/execute command

Command Information Bar

- **One button push to select any command menu**
  - Selected command executed by pressing the same button
- **Settings include all system parameters**
- **Next step guidance on command information bar**

Navigation panel – Run Protocol

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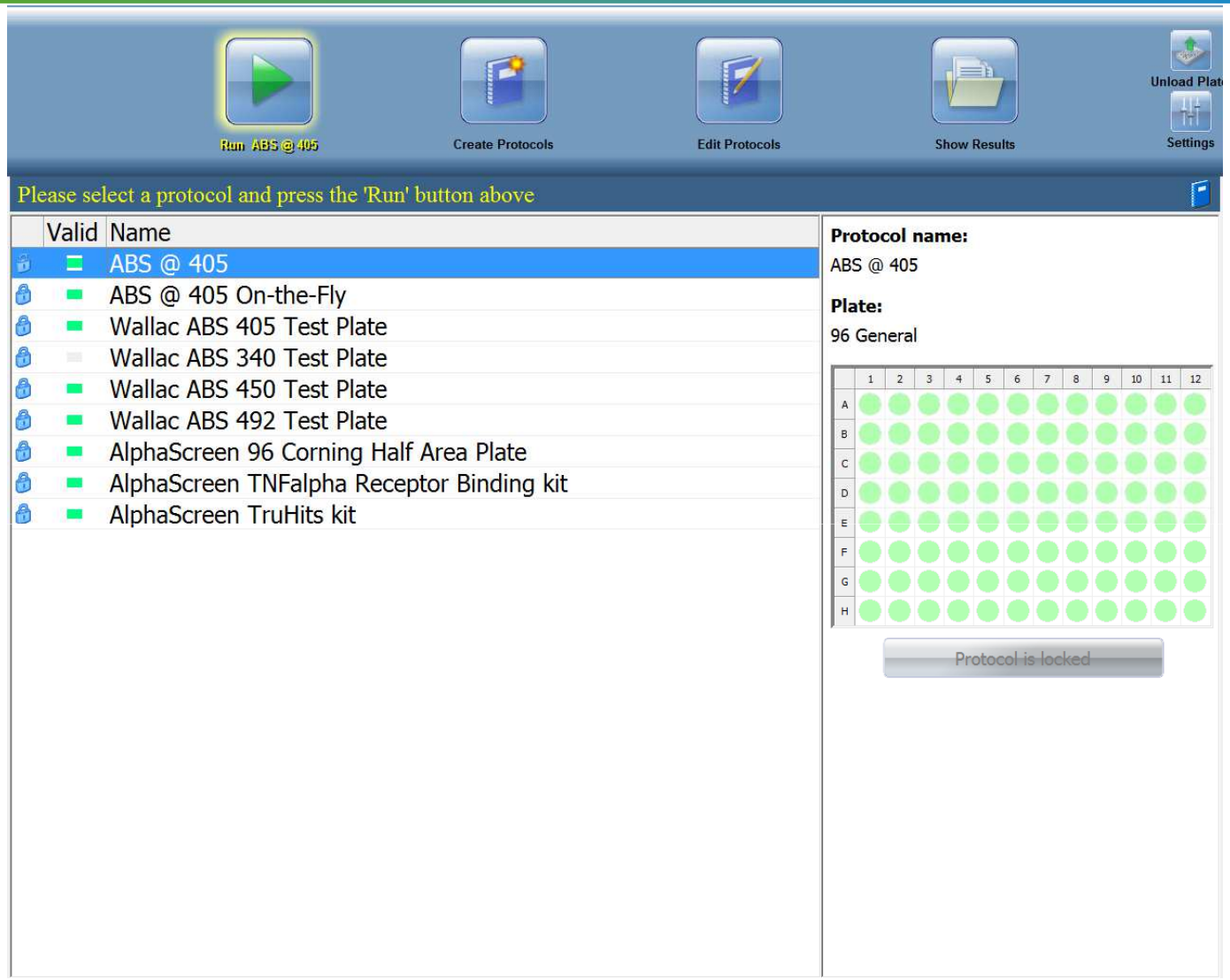
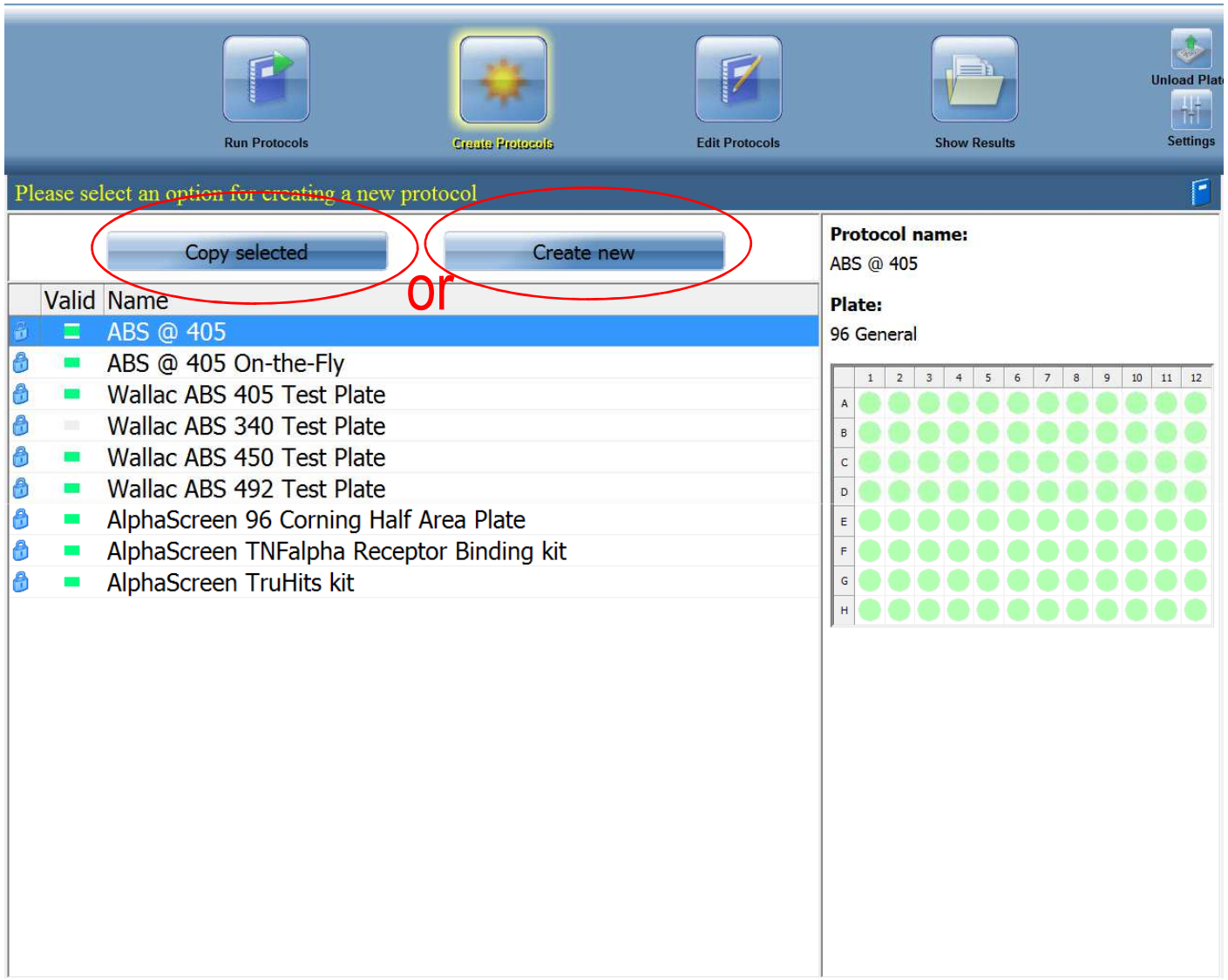
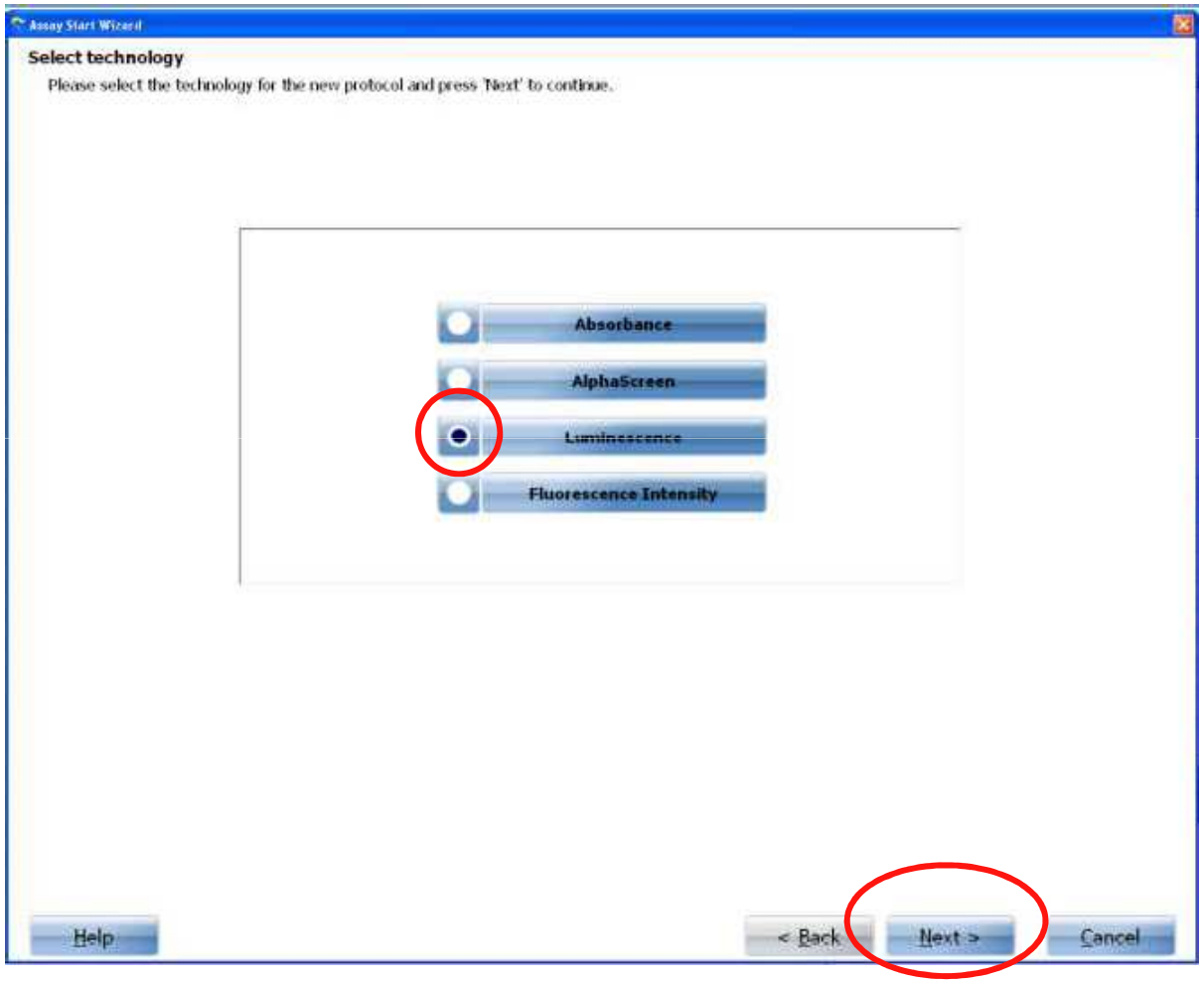


Navigation panel – Create Protocols



Navigation panel – Create Protocols



Navigation panel – Create Protocols

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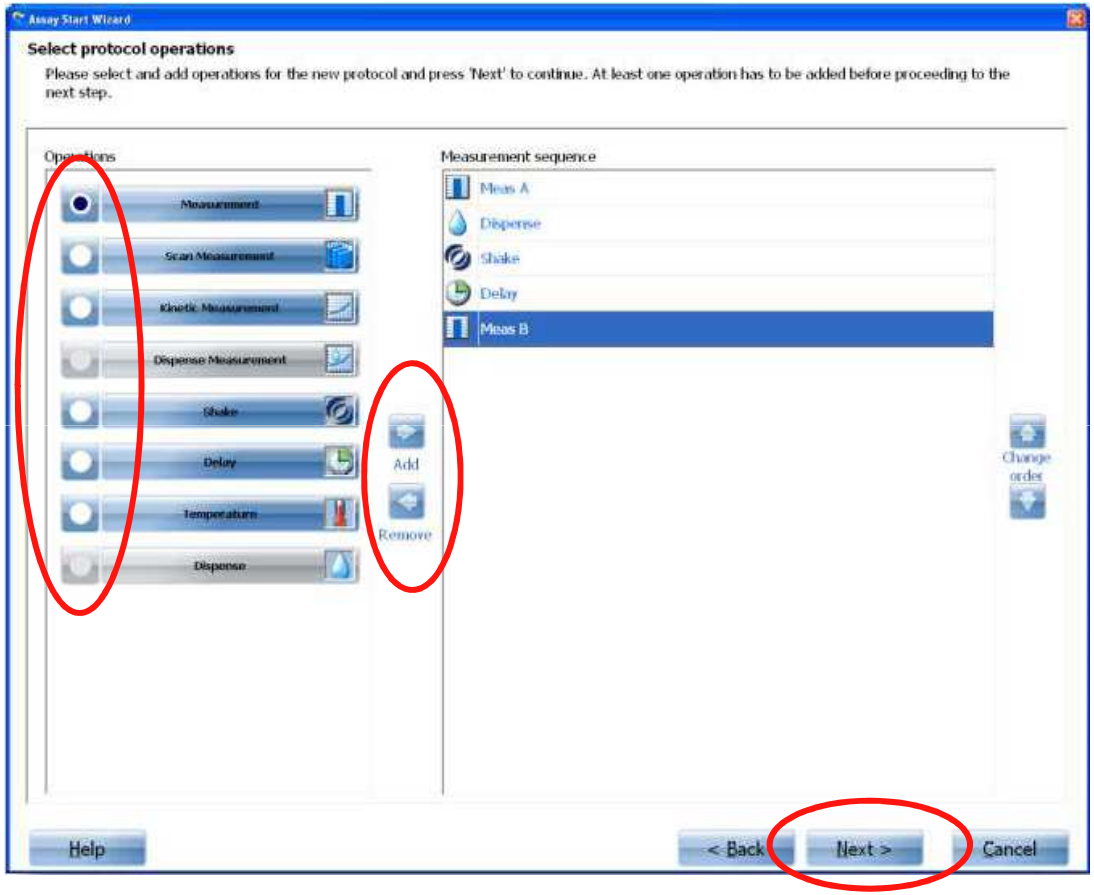

- 從偵測方式中選一個：

  Measurement – 一般偵測

  Scan Measurement – 多點偵測

  Kinetic Measurement – 連續偵測

  On-the-fly Measurement – 快速模式

  Wavelength Scan Measurement – 掃瞄樣品吸收光譜功能

- 再選擇是否

  Shake – 震盪微量盤

  Delay – 等待時間

- 選擇時按 Add 將選項放入 Measurement sequence 內，取消時使用 Remove 移除。

  先加入動作，再將不需要的移除！

- Measurement sequence 內順序以上下箭頭變動。

Navigation panel – Create Protocols

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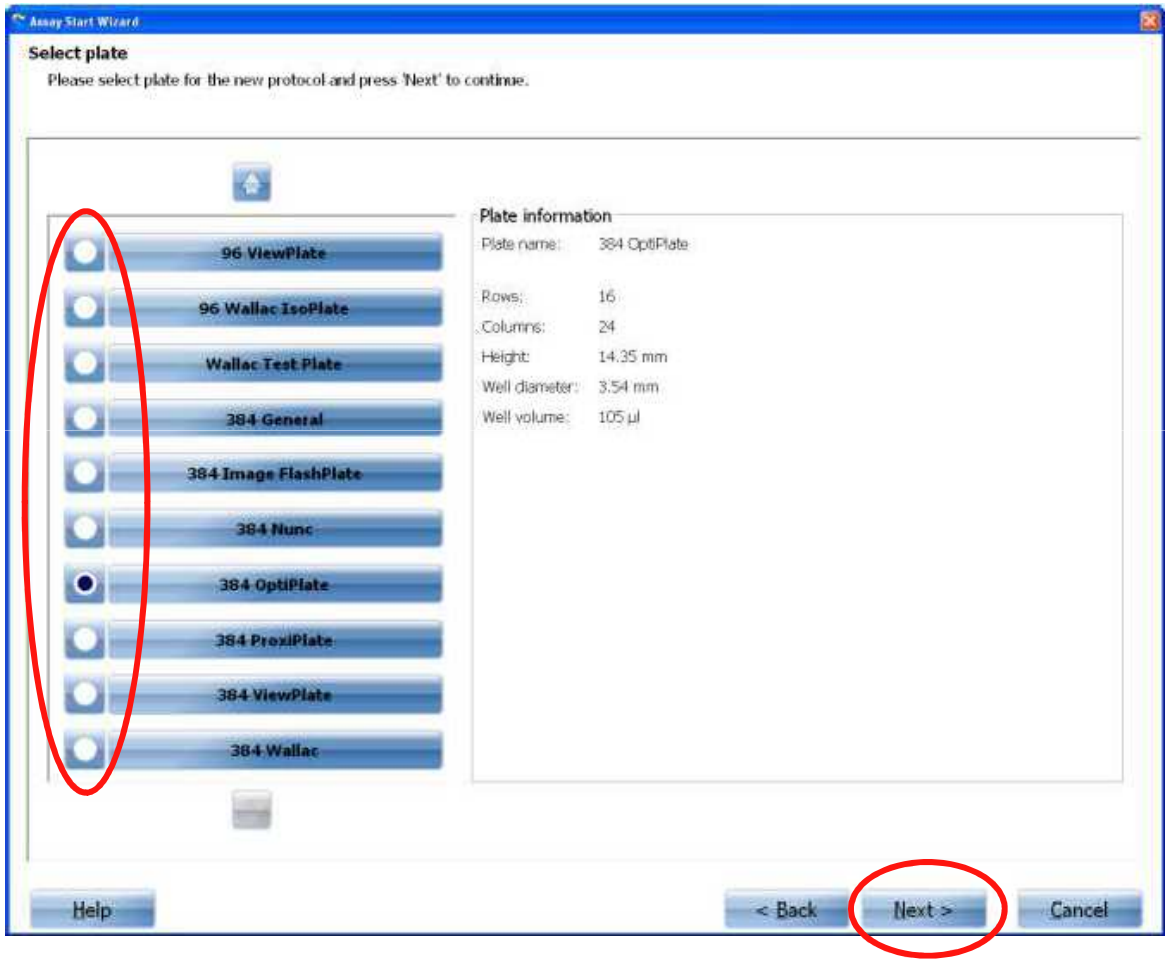
Choose plate type



✱ add new plate type：

Settings - Inventory - Plates

Navigation panel – Create Protocols

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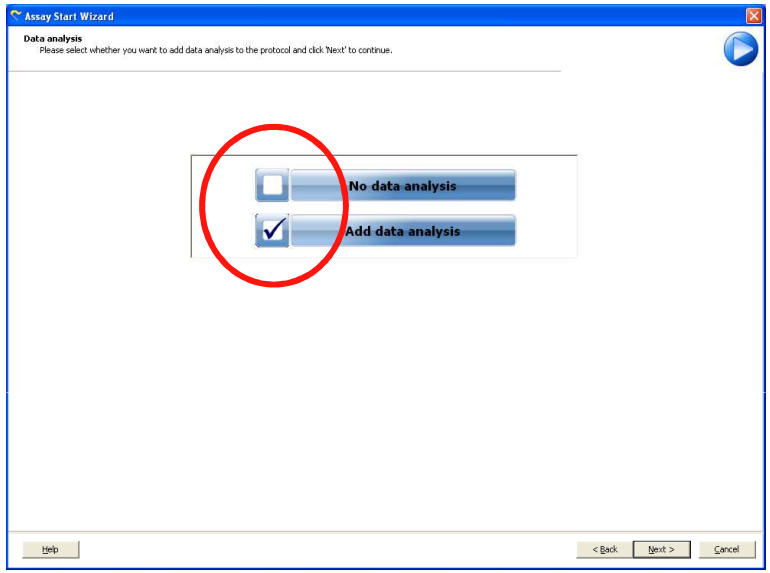
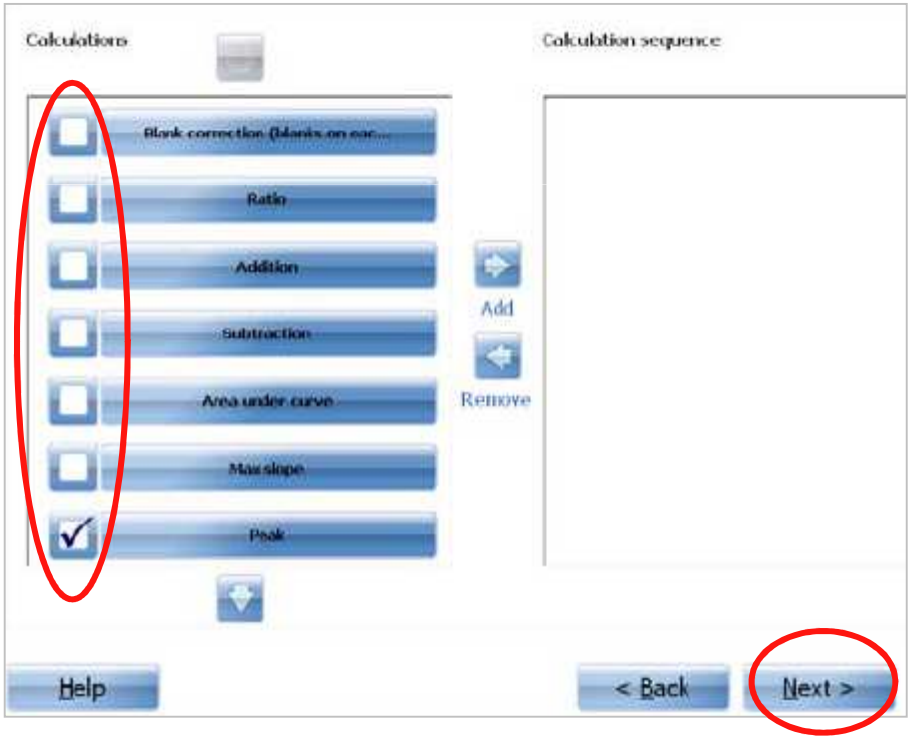
Data analysis





Navigation panel – Create Protocols

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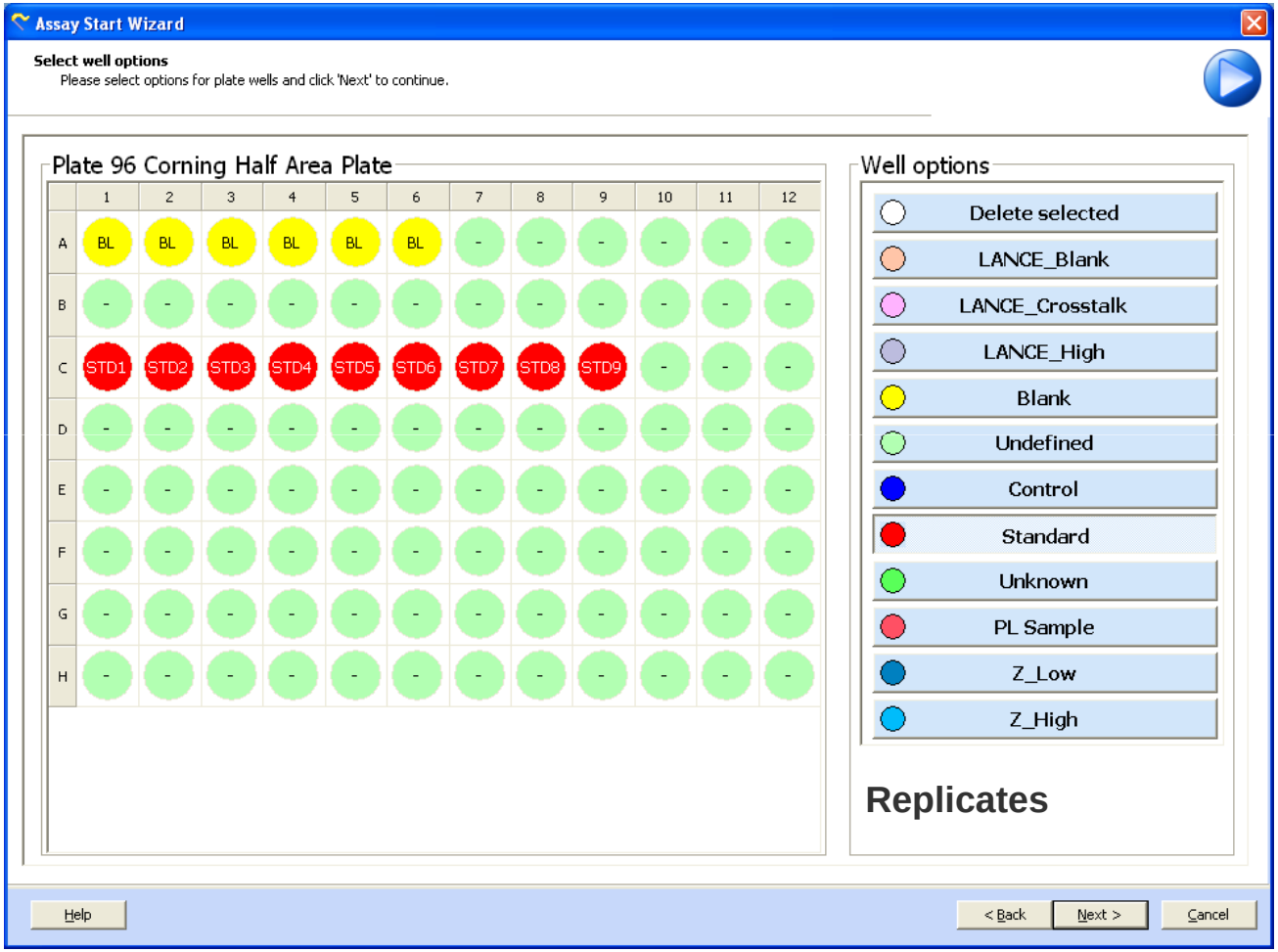
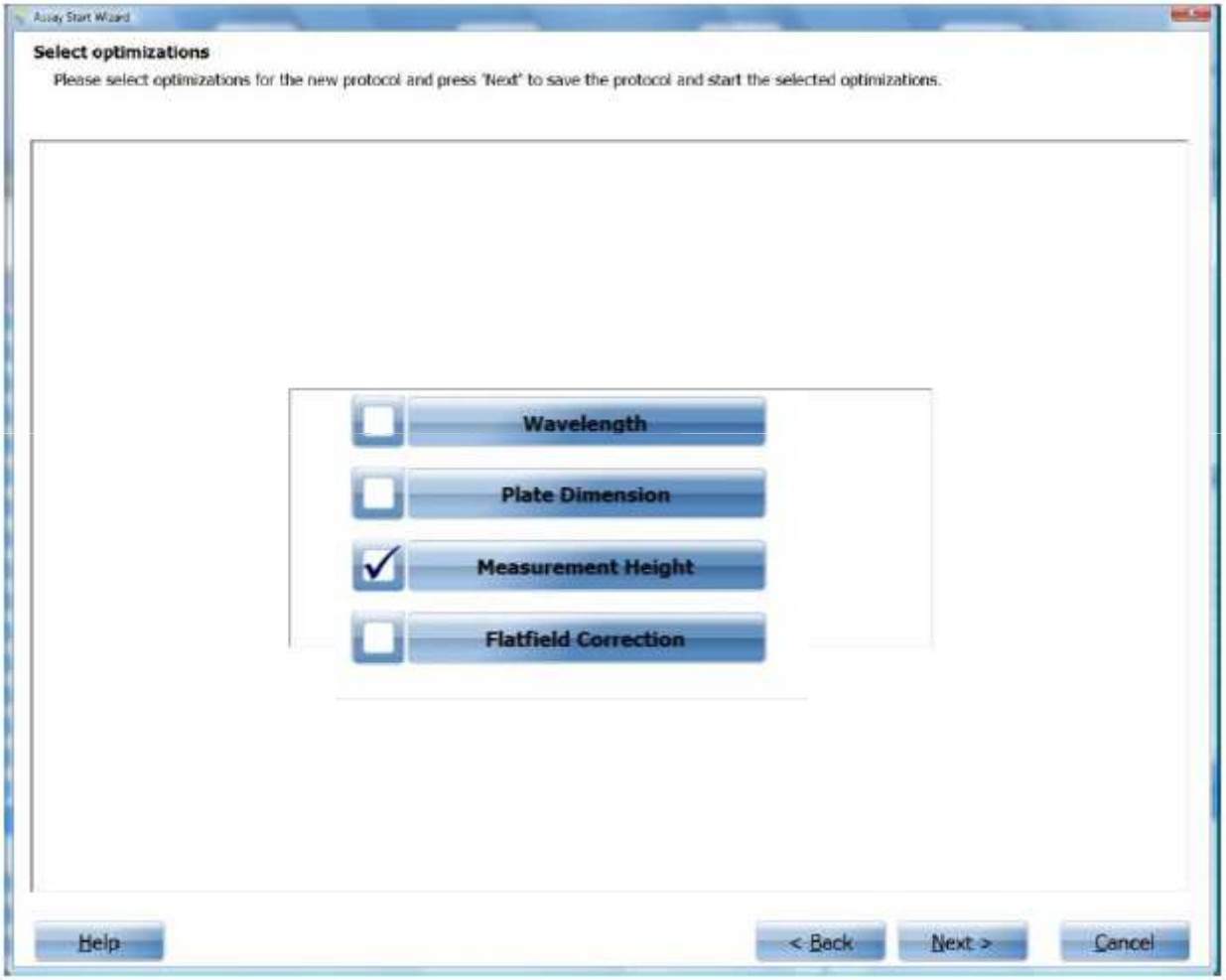
Select well options



Select optimizations



Navigation panel – Create Protocols

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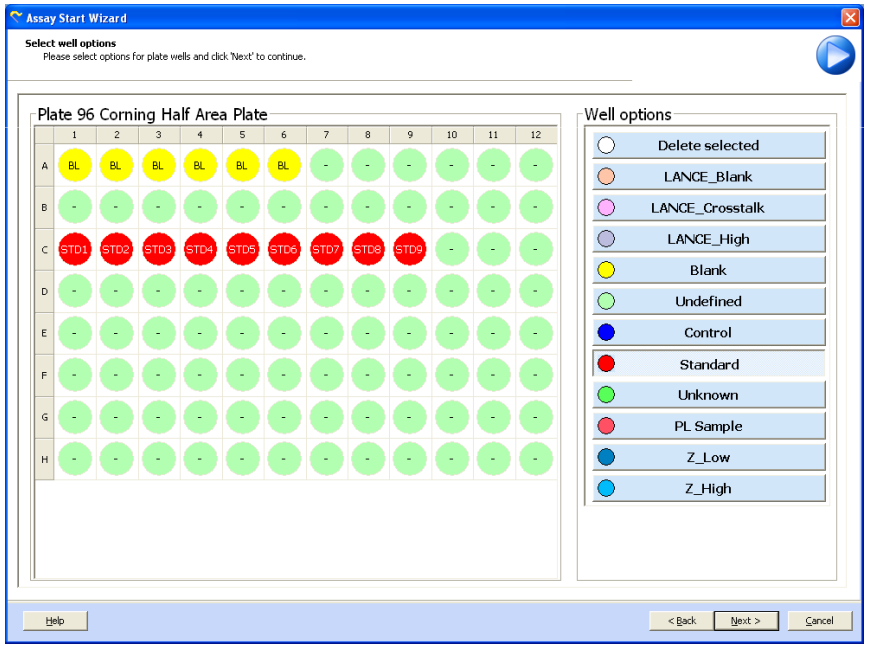
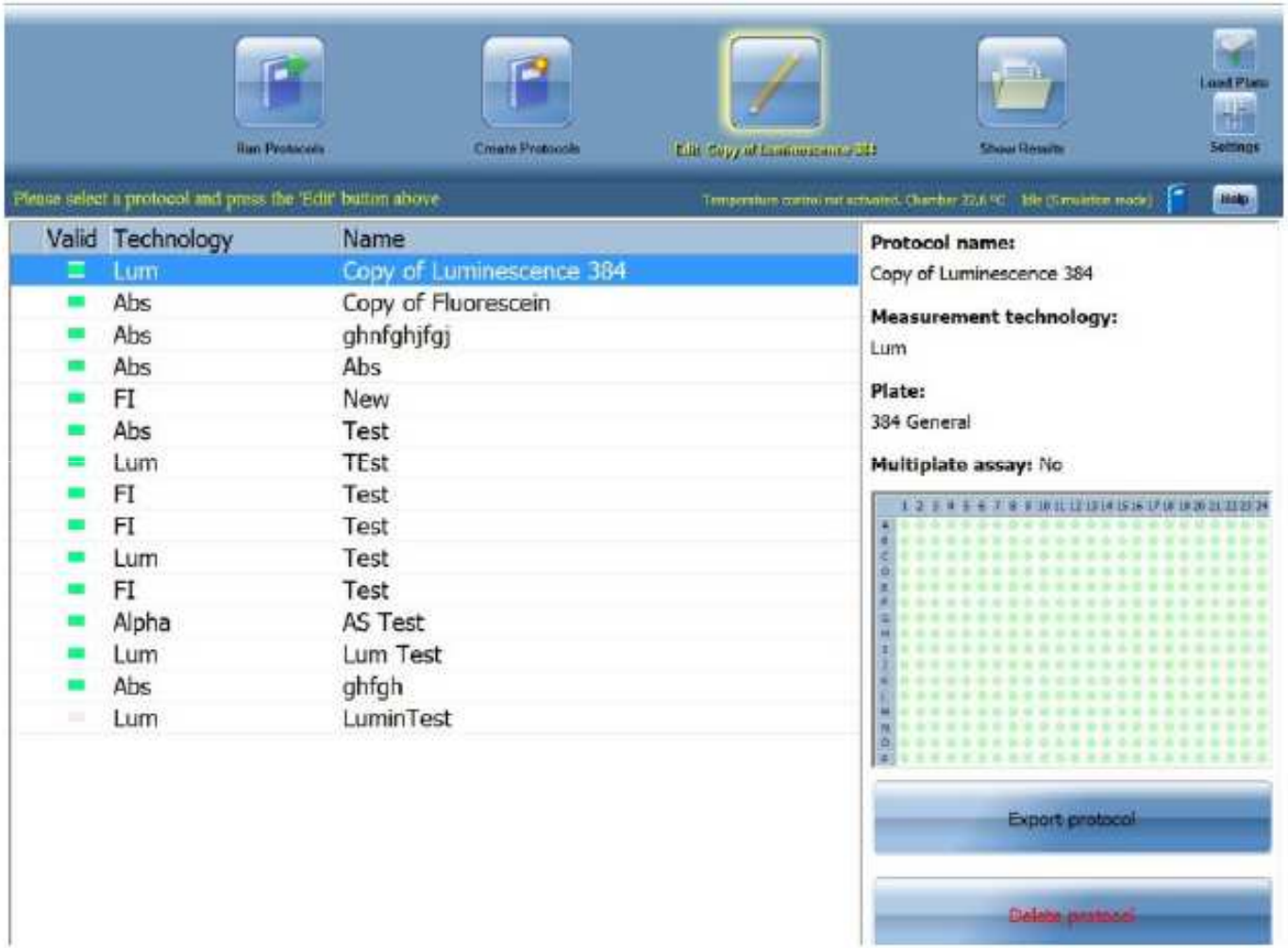
Save Optimization



Navigation panel –Edit Protocols



Navigation panel – Results

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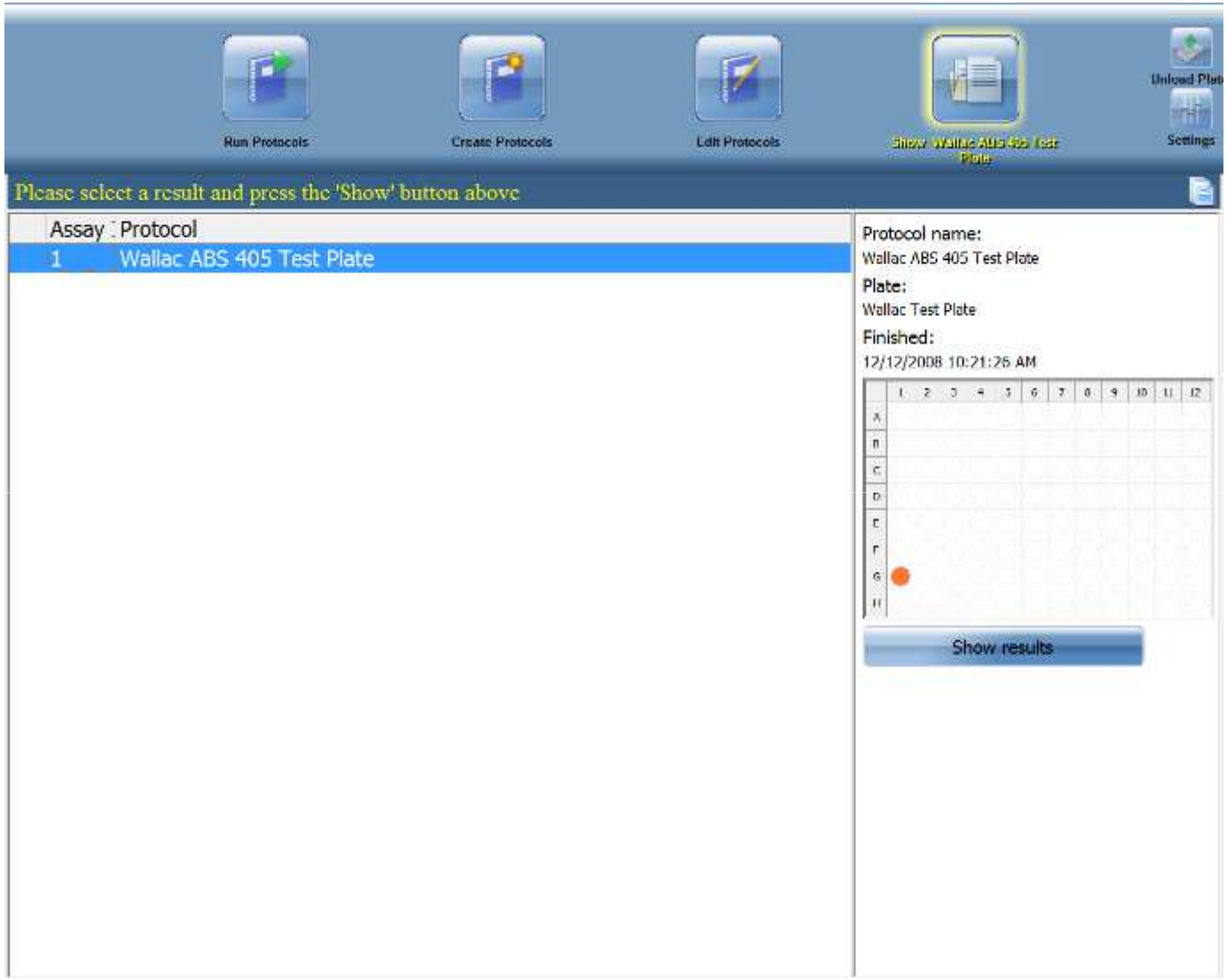

Navigatin panel – Settings…options

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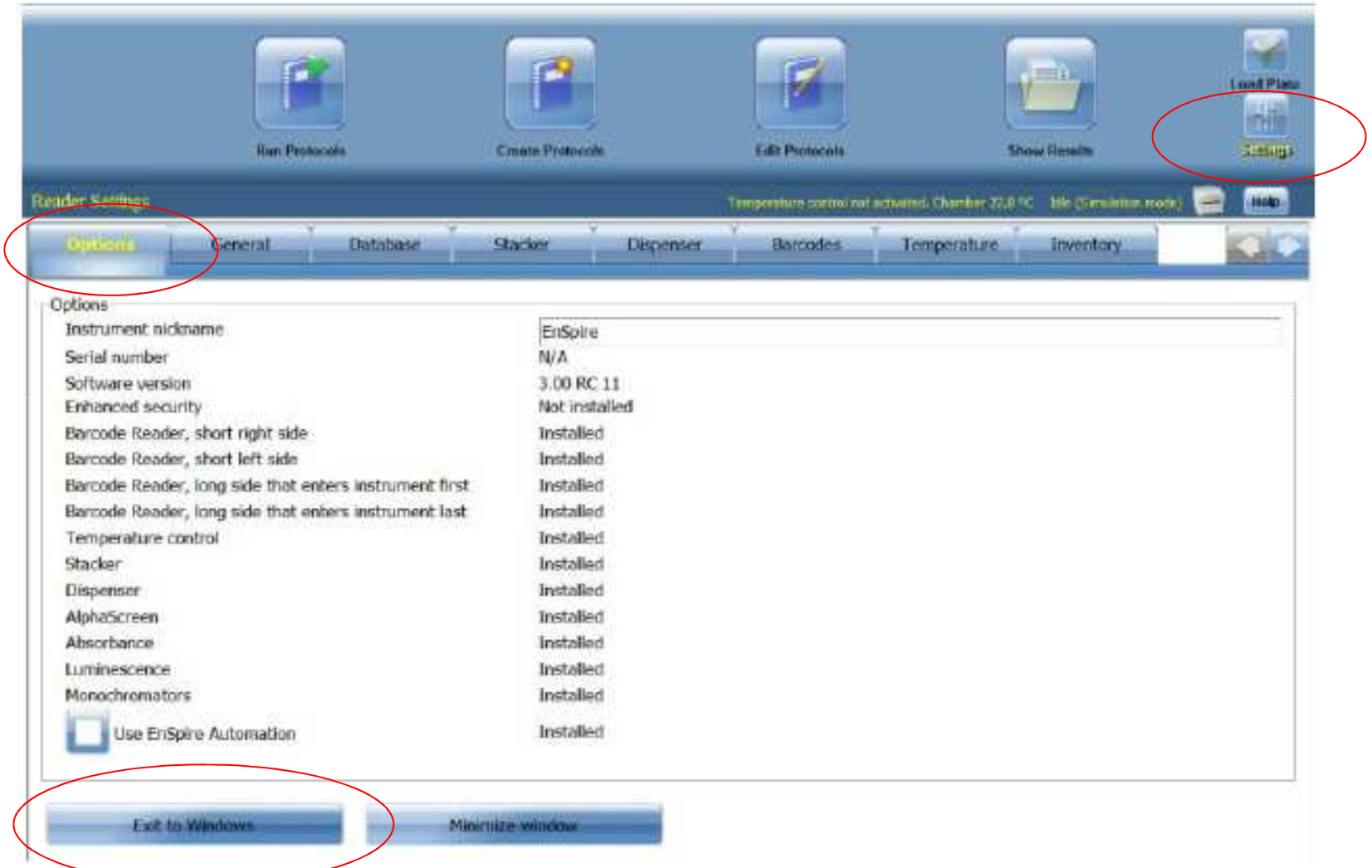
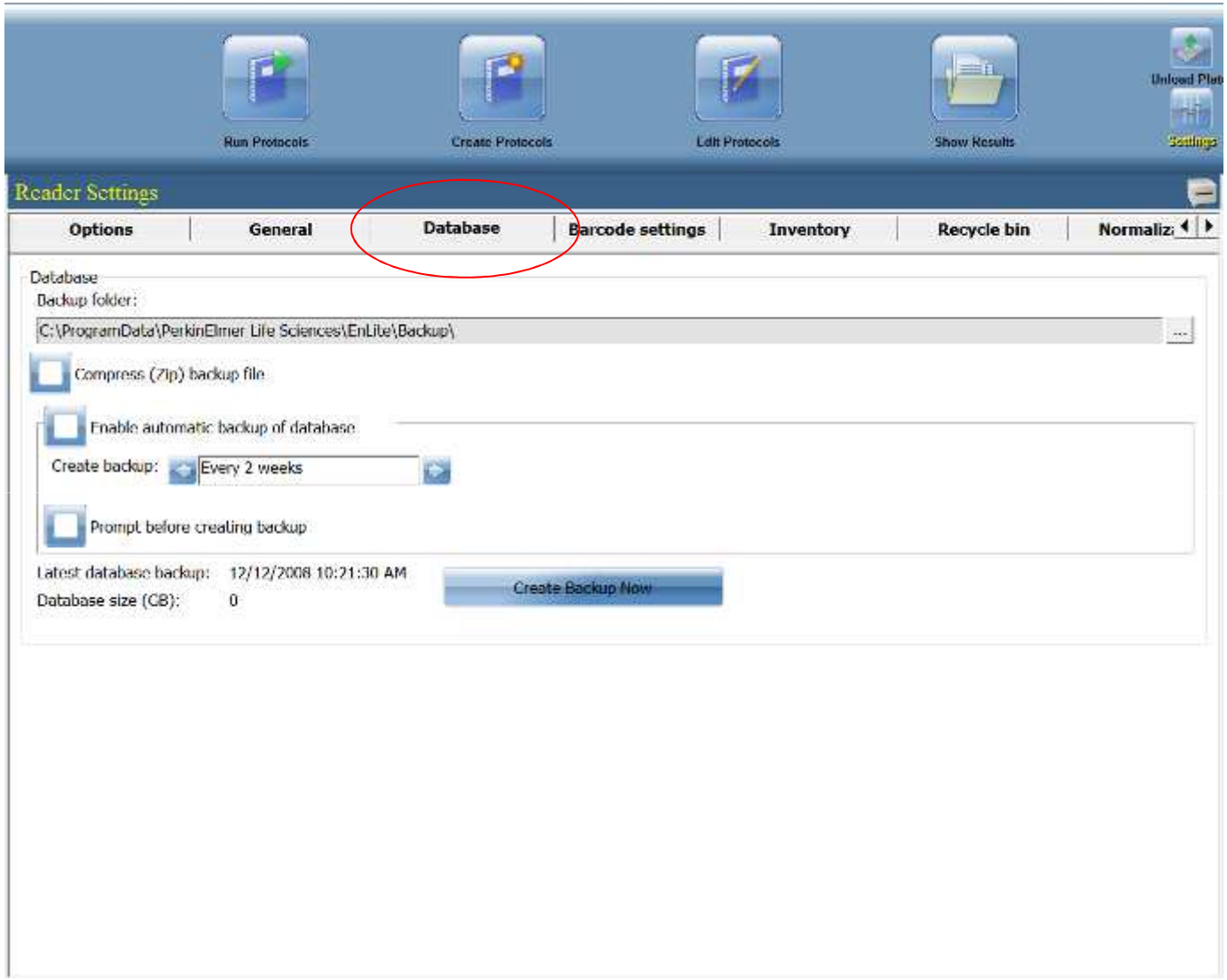


Settings…database



Settings…barcode settings

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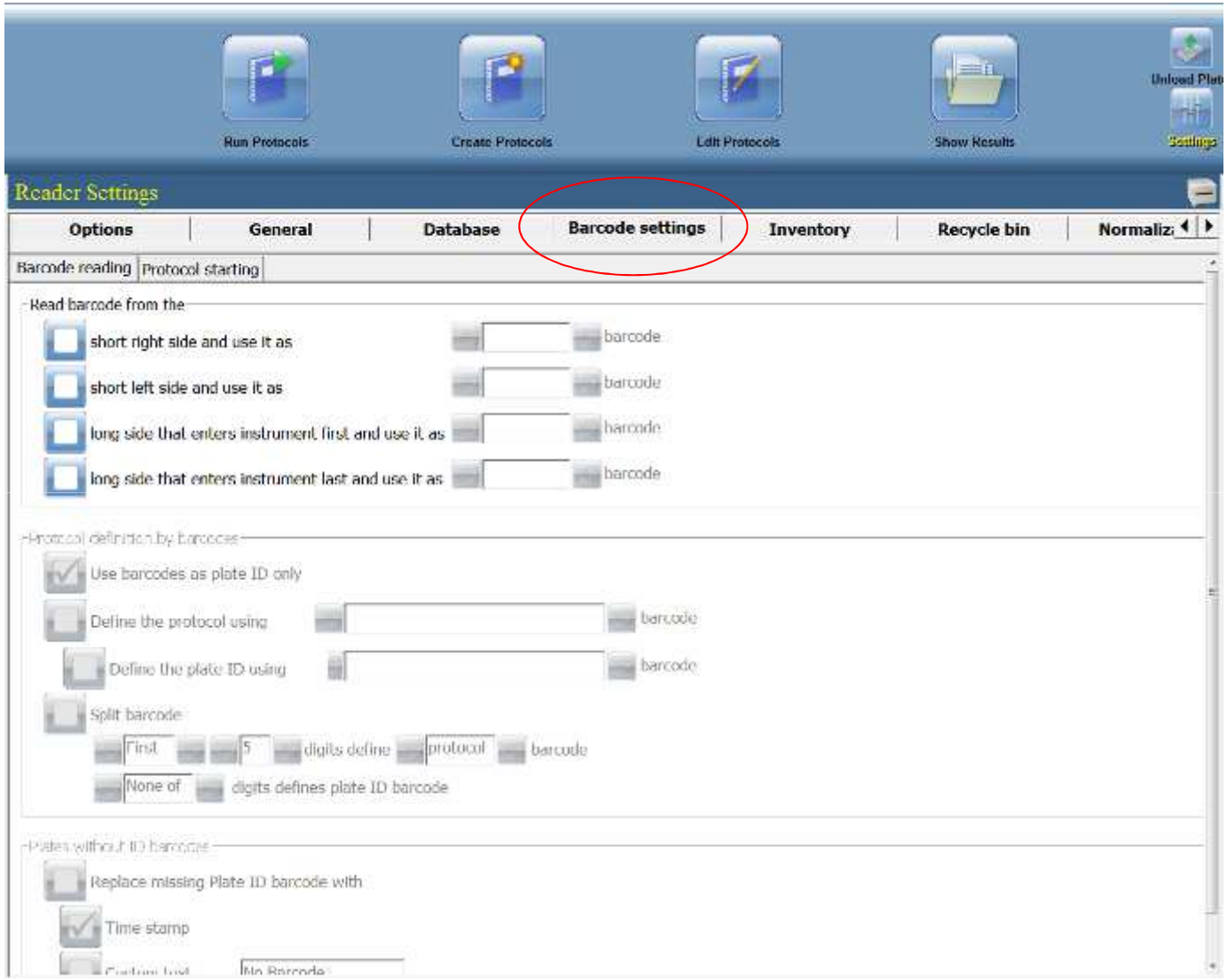
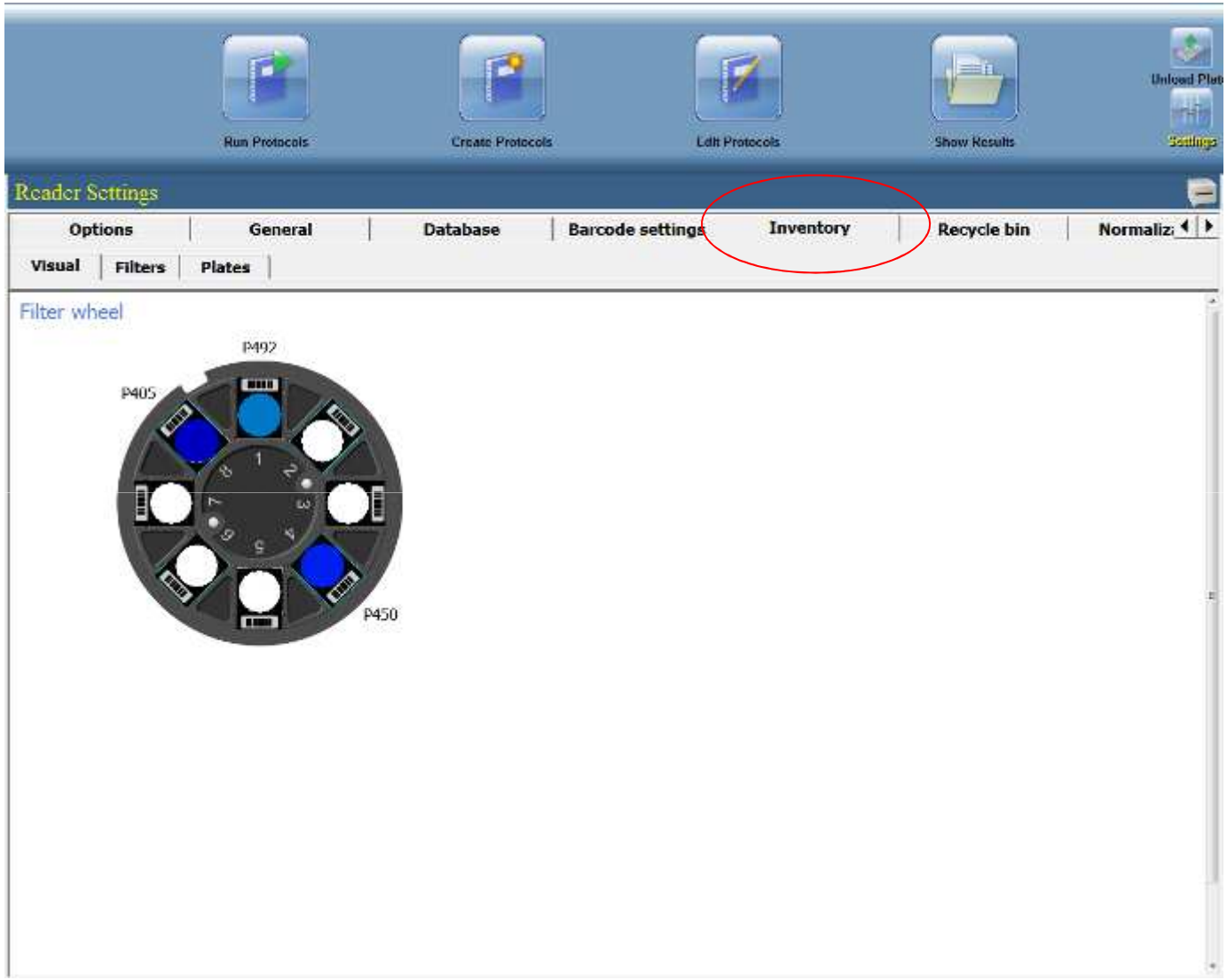


Settings…inventory



THANK YOU