amsbio

TREVIGEN®

Second Generation PARP1 Pharmacodynamic Assay

What is a Pharmacodynamic Assay?

- Pharmacodynamic Assays:
  - Provide evidence of drug action on molecular target.
  - Guide drug development process.
  - Base line values may be used to stratify patient response to therapy.

PARP1

- In response to many types of DNA damage PARP1 polymerizes polymers of ADP-ribose on to itself and other proteins.
- These polymers serve as scaffold to recruit other repair proteins to the site of damage to repair the damaged DNA.

Action of PARP1 and PARG

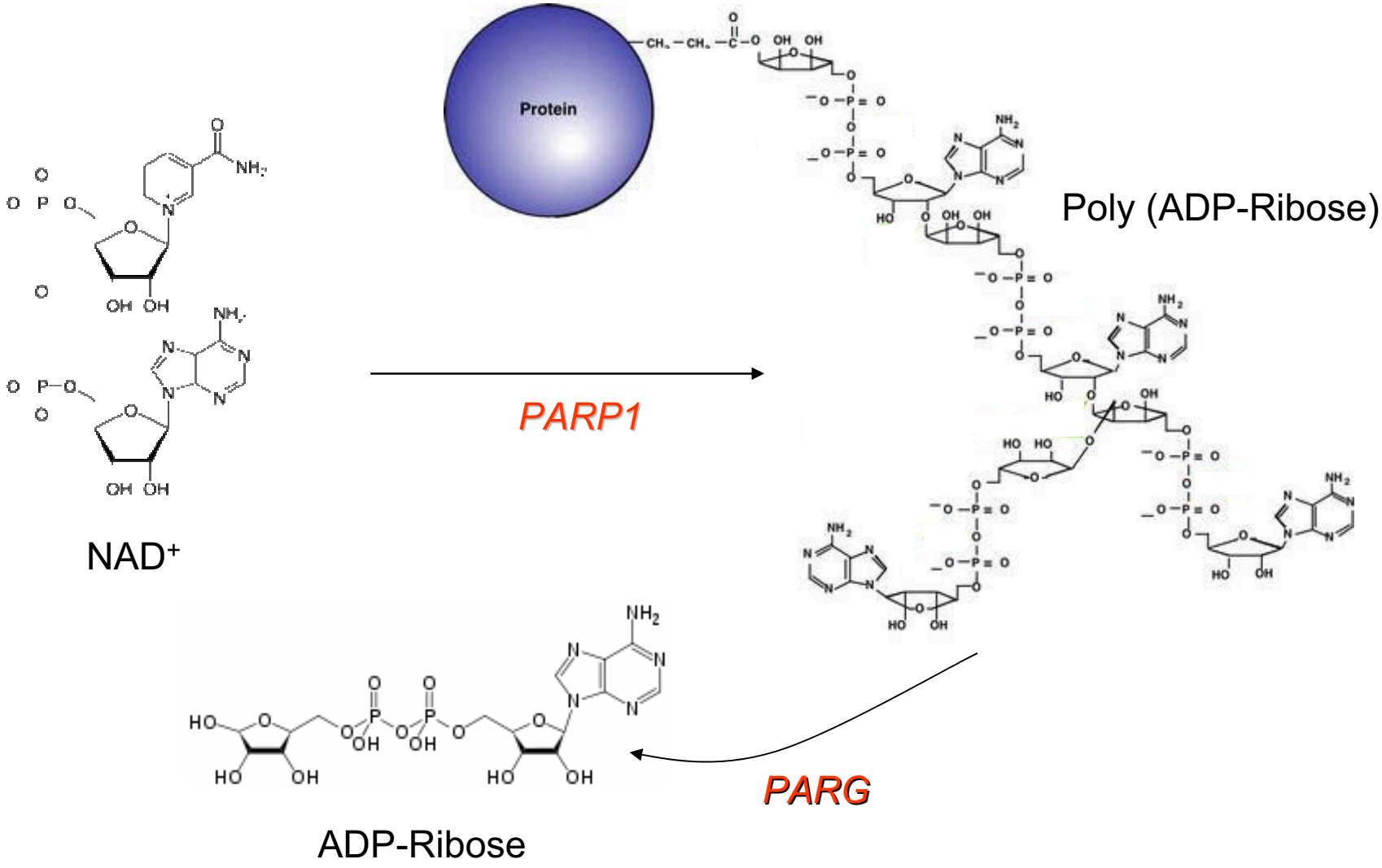


Figure 1.

Three important points to understand About PARP1 and DNA Repair

- PARP1 Inhibition results in the accumulation of single strand DNA breaks.
- As a result of the replication process these single strand breaks are converted to double strand breaks.
- Unrepaired double strand breaks are lethal to the cell
- Double strand breaks are repaired by Homologous Recombination which requires BRCA1/2.

Cells Deficient in BRCA1/2 (Homologous Recombination) are Sensitive to PARP1 Inhibitors

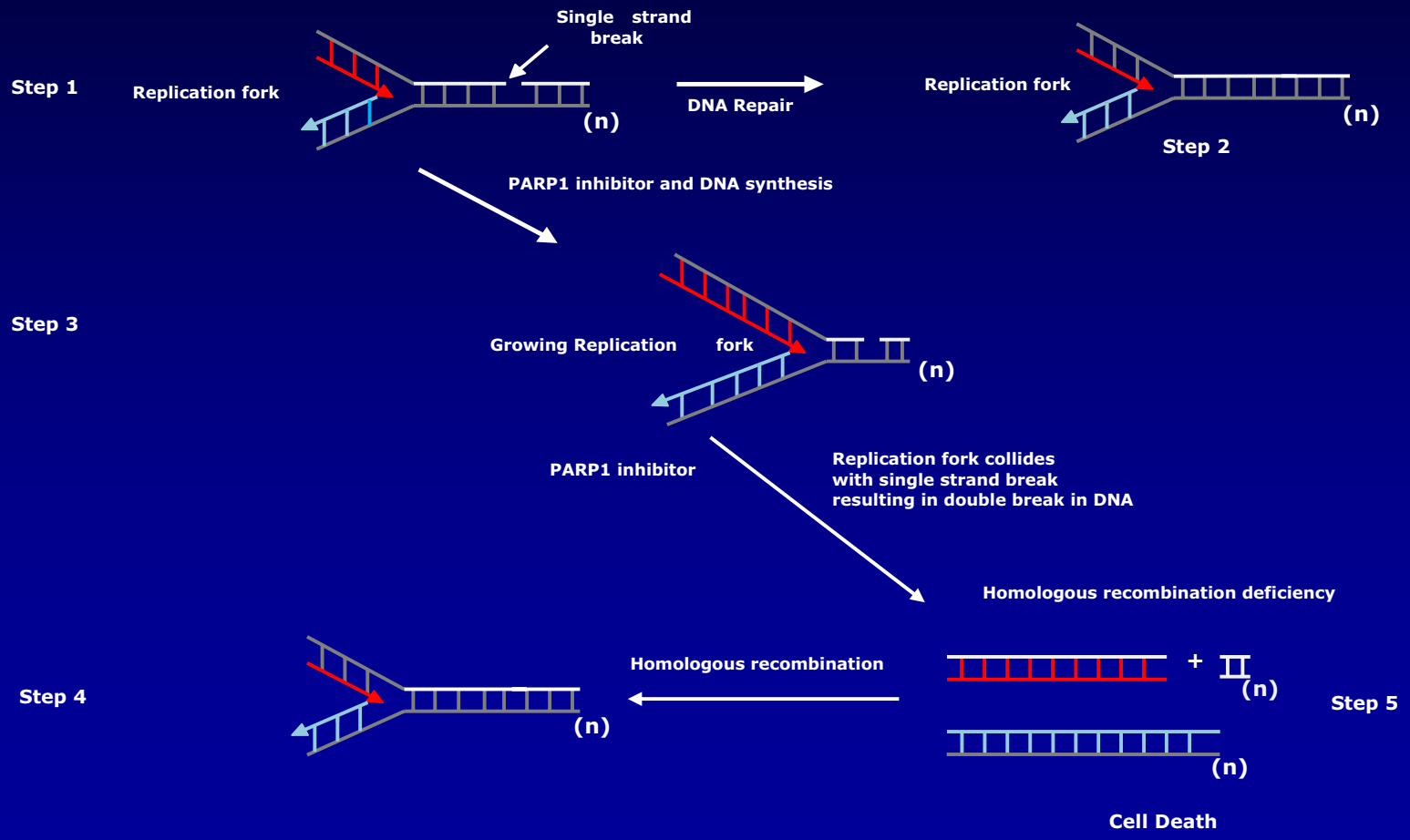
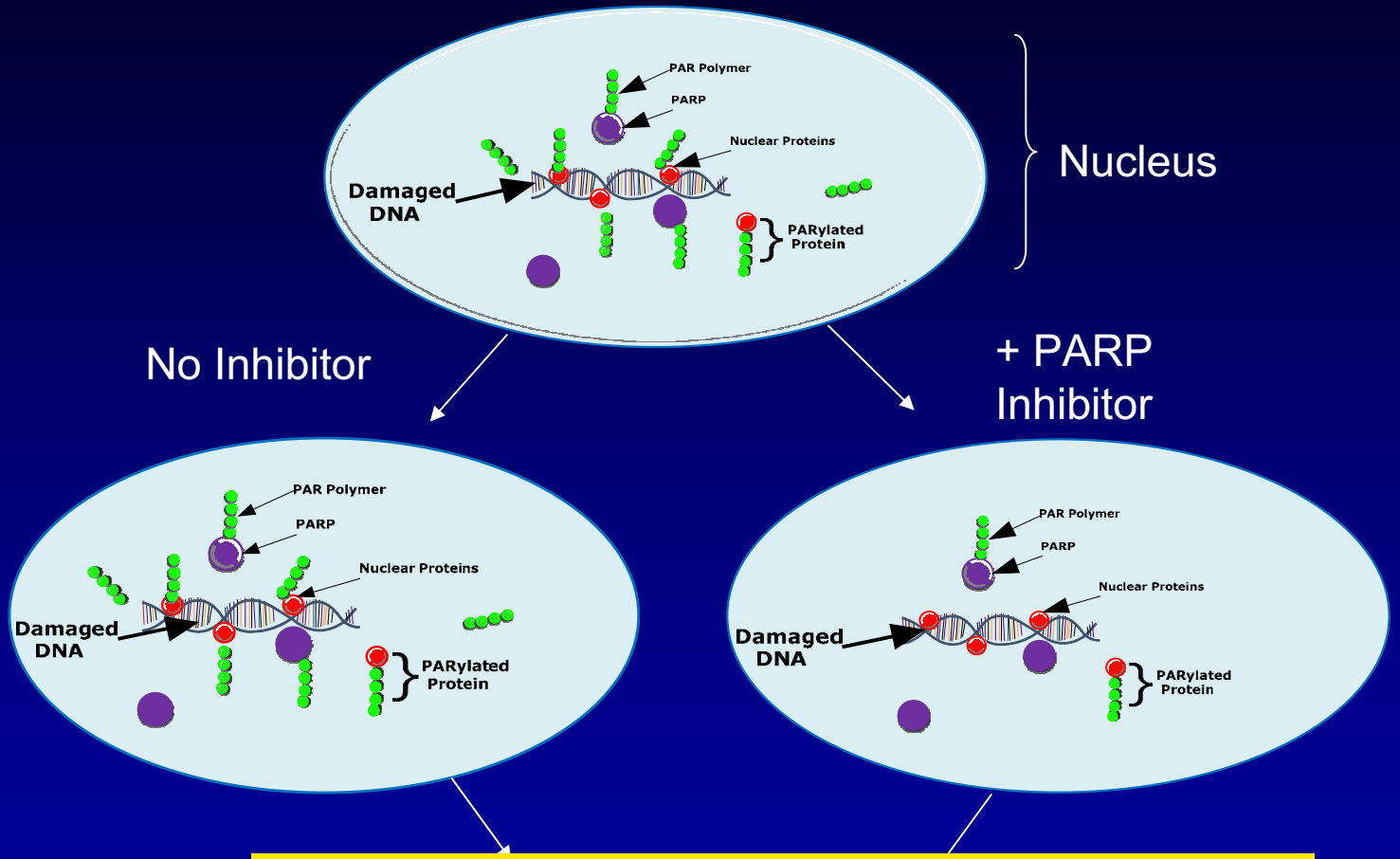


Figure 3.

Clinical Significance of PARP1 and BRCA1/2

- BRCA1/2 has been identified as the familial "breast cancer genes"
- Many other breast tumors have low levels of BRCA1/2
- Breast Tumors deficient in BRCA1/2 are sensitive to PARP1 inhibitors.

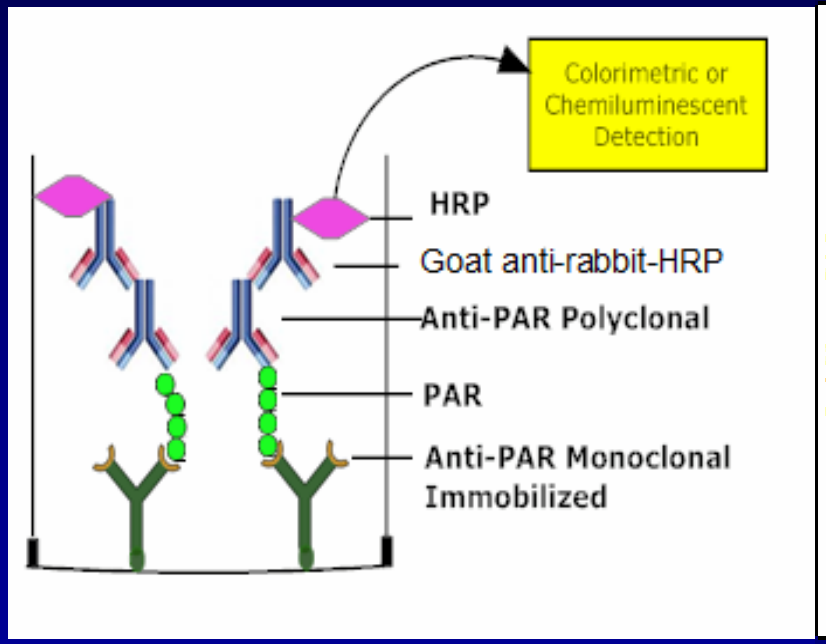
PARP Pharmacodynamic Assay

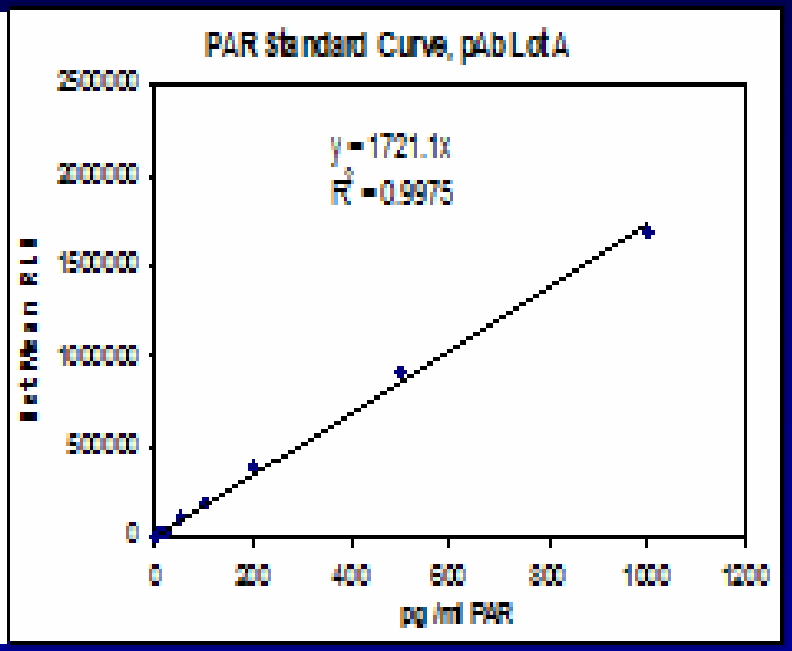


Determine if inhibitor changed PARP activity in vivo using a Pharmacodynamic Assay

Figure 4.

PARP1 Pharmacodynamic Assay



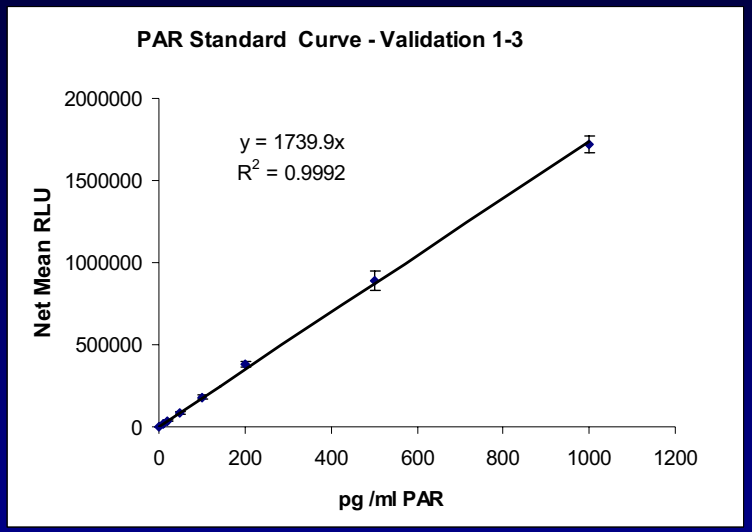


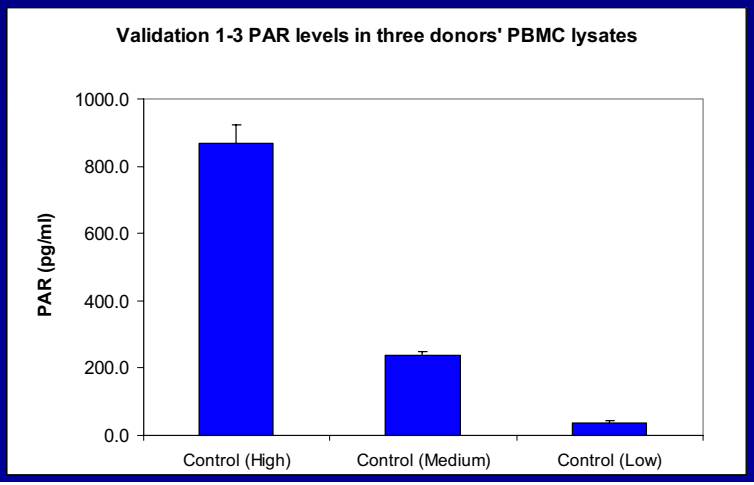
The Assay Provides Reproducible Results

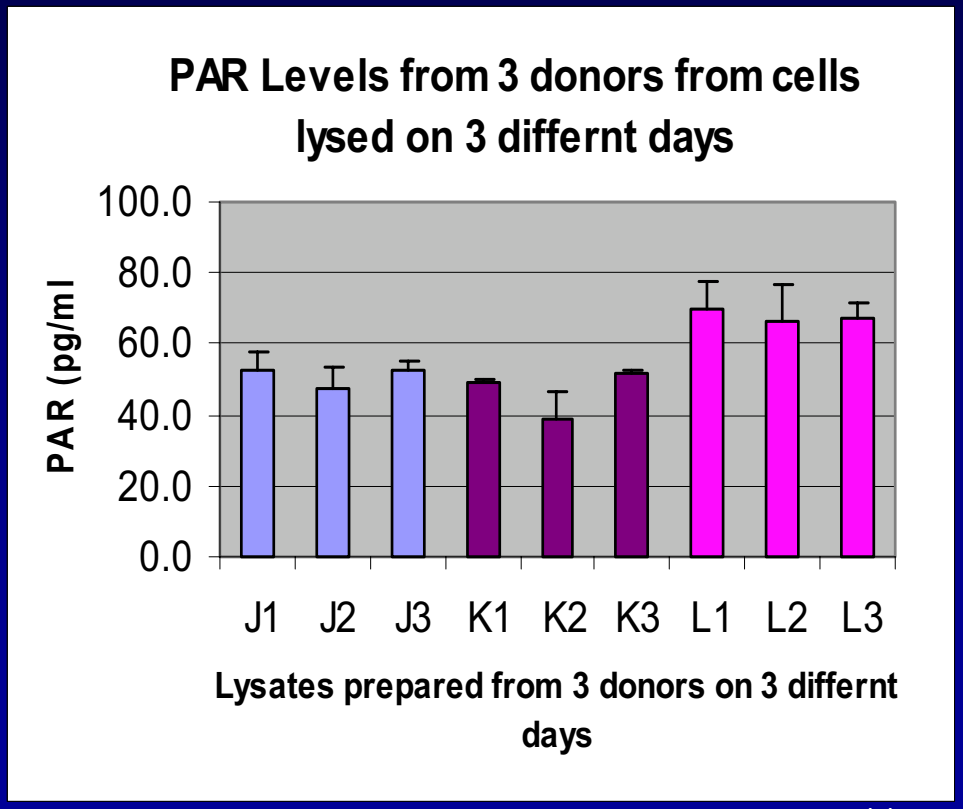
- Blood was drawn from three volunteers. White cells were prepared. Aliquoted and frozen. Lysates were made at three different days.
- Reagents were tested three times over a period of two weeks (figures 7-9).
- Jurkat Cell lysates containing high (750-1150 pg/ml) , medium (150-300 pg/ml) and low (20-60 pg/ml) three levels of PAR used as positive control.
- Statistical analysis to determine performance of the assay over a two week period.

The Assay Provides Reproducible Results

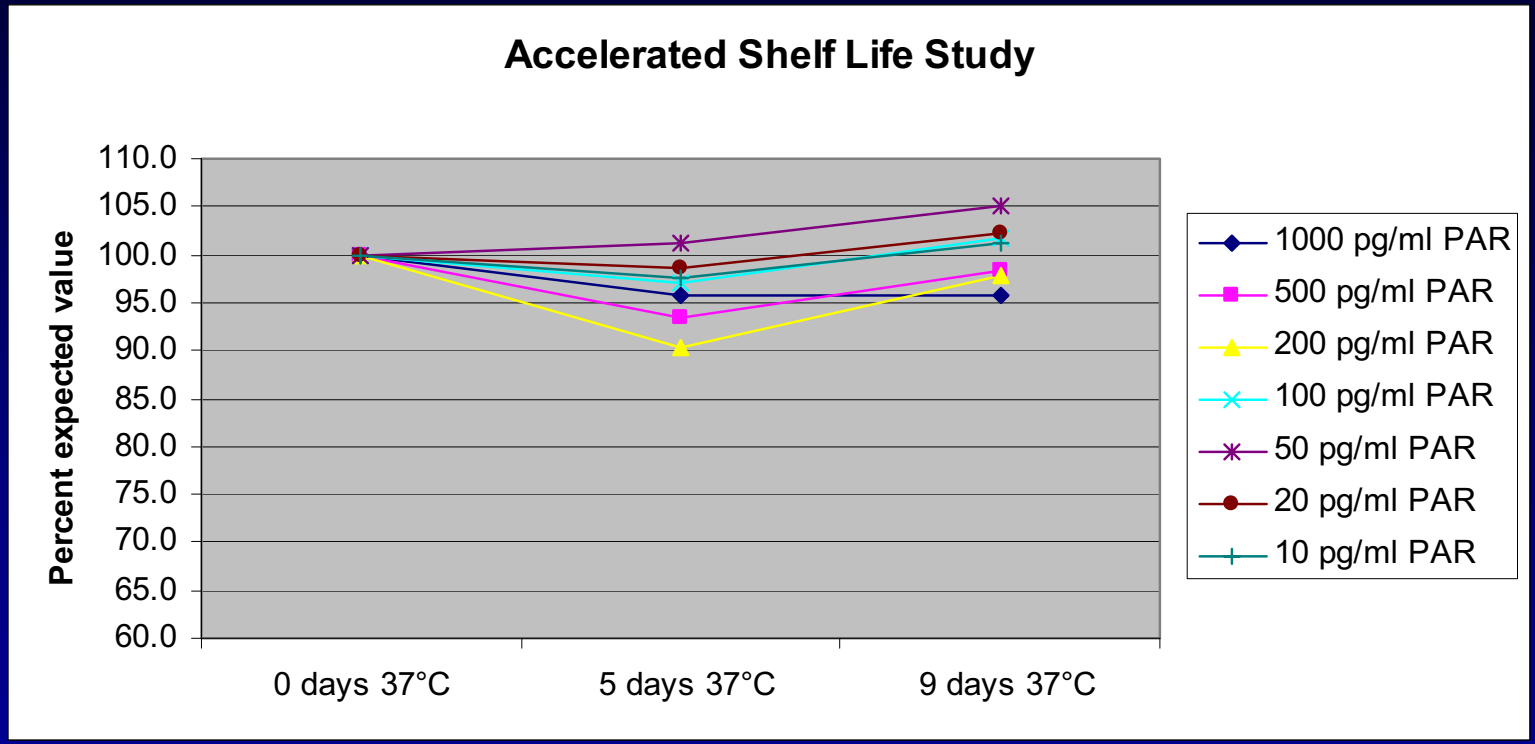
Validation 1-3







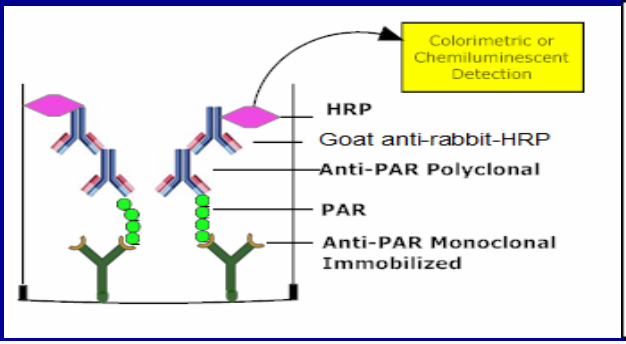
Accelerated Shelf Life Study on Coated Plates

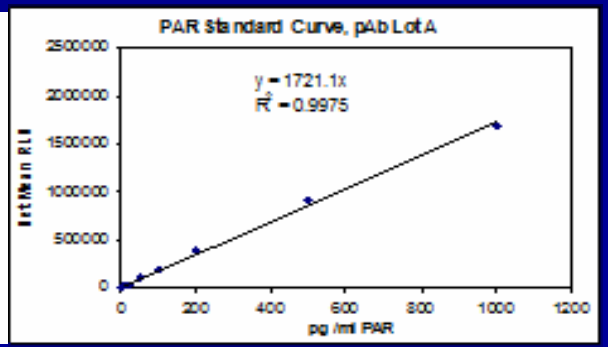


Coated plates are stable for at least 1 year

PDA Kit Components

Part #	Component	Size
4520-096-01	PAR Standard, 25 pg/µl	5 x 20 µl
4520-096-02	Sample Buffer	20 ml
4520-096-03	PAR Polyclonal Detecting Antibody	30 µl
4520-096-04	Goat anti-Rabbit IgG-HRP	30 µl
4675-096-01	PARP PeroxyGlow™ A	6 ml
4675-096-02	PARP PeroxyGlow™ B	6 ml
4520-096-05	Cell Lysis Reagent	30 ml
4520-096-06	DNase 1, 2 Units/µl	60 µl
4520-096-07	100X Magnesium Cation	500 µl
4520-096-P	Pre-coated white 96-stripwell plate, and 5 sealers	1 plate
4520-096-08	Jurkat Cell Lysate Standard Control, Low	600 µl
4520-096-09	Jurkat Cell Lysate Standard Control, Medium	600 µl
4520-096-10	Jurkat Cell Lysate Standard Control, High	600 µl
4520-096-11	Antibody Diluent	15 ml
4520-096-12	20% (w/v) SDS	1 ml





Synthetic Lethality