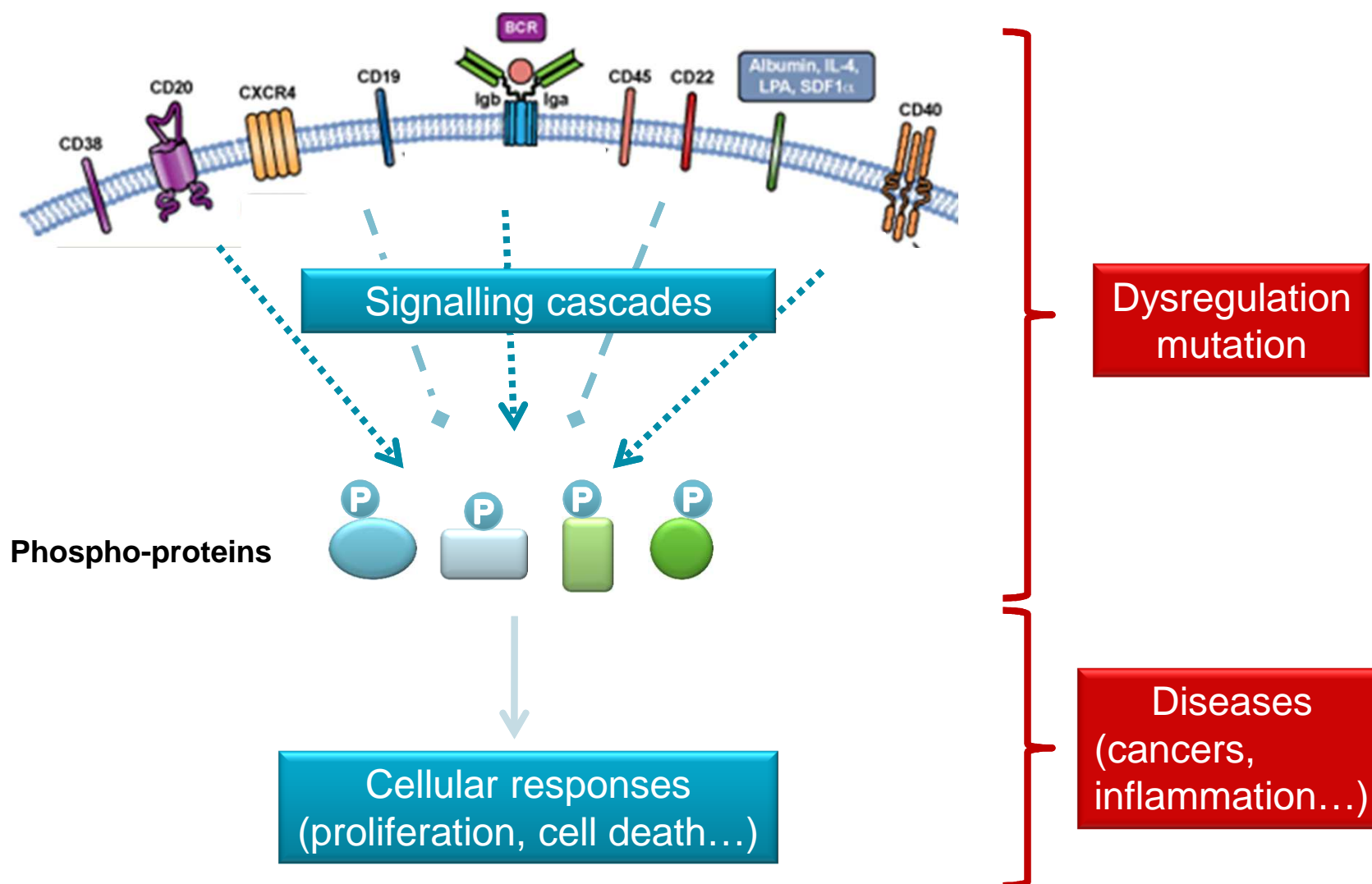


**INVESTIGATING KINASE  
ACTIVITY IN A CELLULAR  
CONTEXT**



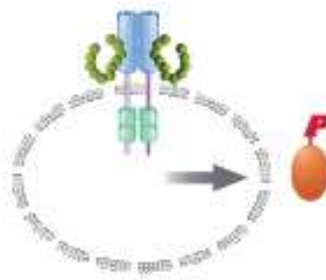
*E. Trinquet  
VP Product Development  
SLAS meeting, Cisbio Tutorial  
January 2013, Orlando*

## Protein phosphorylations are key signalling events

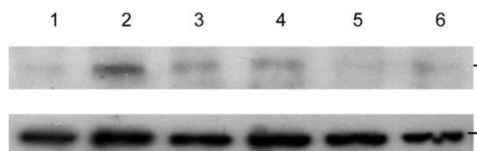


# Common techniques to monitor protein phosphorylation

Cell lysates



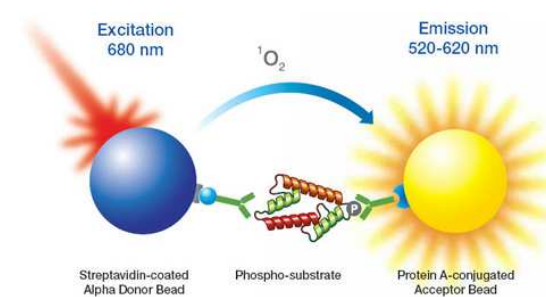
**Western Blot**



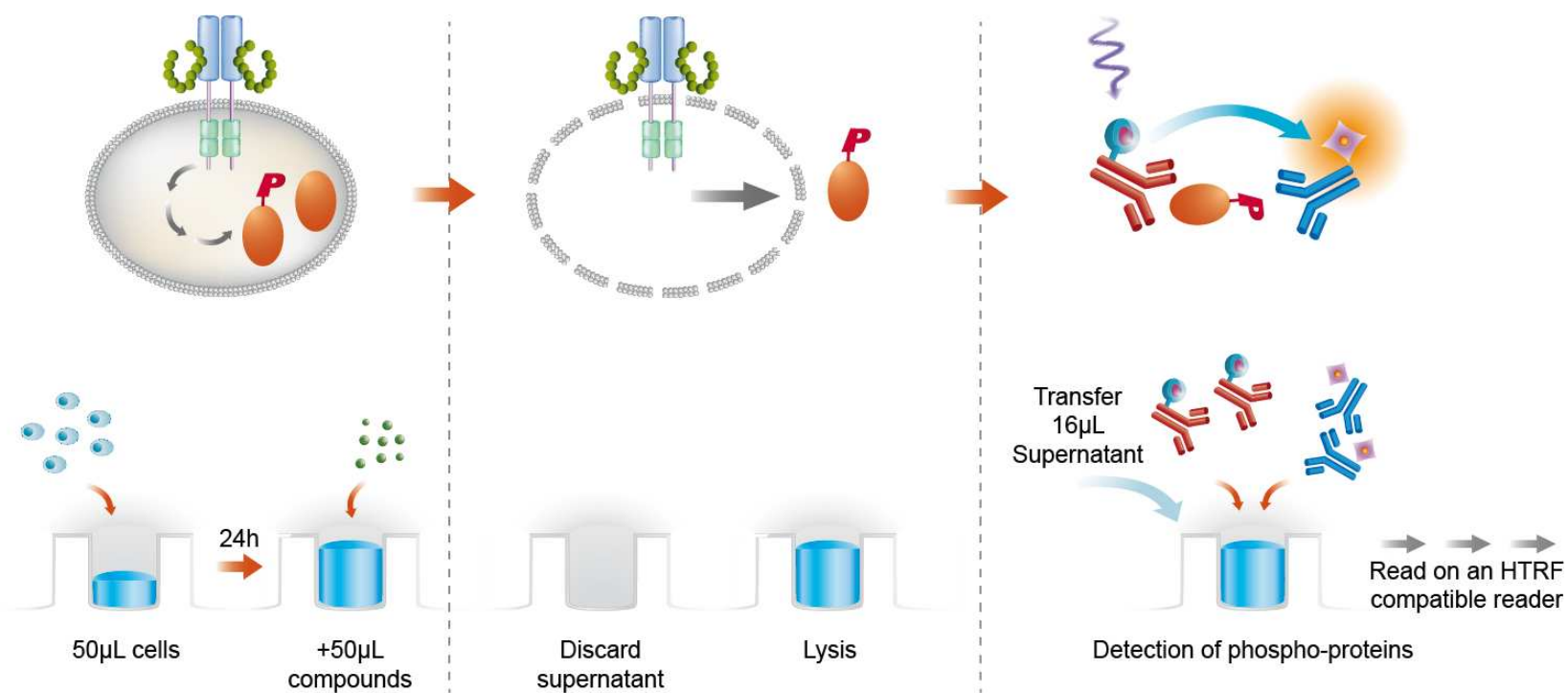
**ELISA  
ELISA-like techniques**



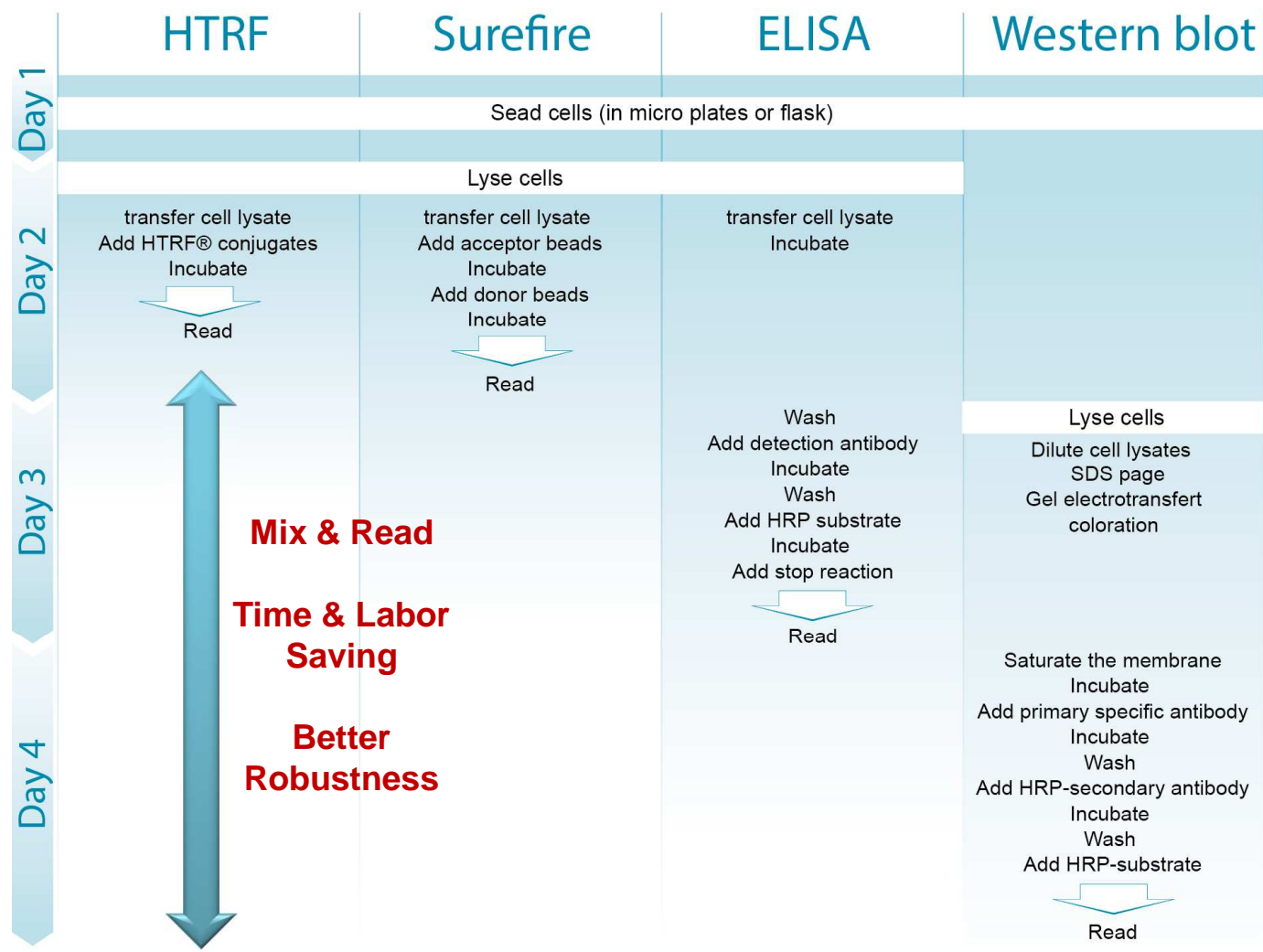
**Homogeneous  
bead-based techniques**



## A straightforward HTRF<sup>®</sup> assay platform to monitor protein phosphorylation using adherent or suspended cells

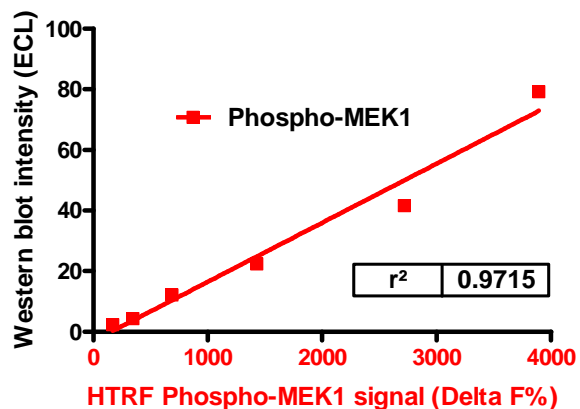
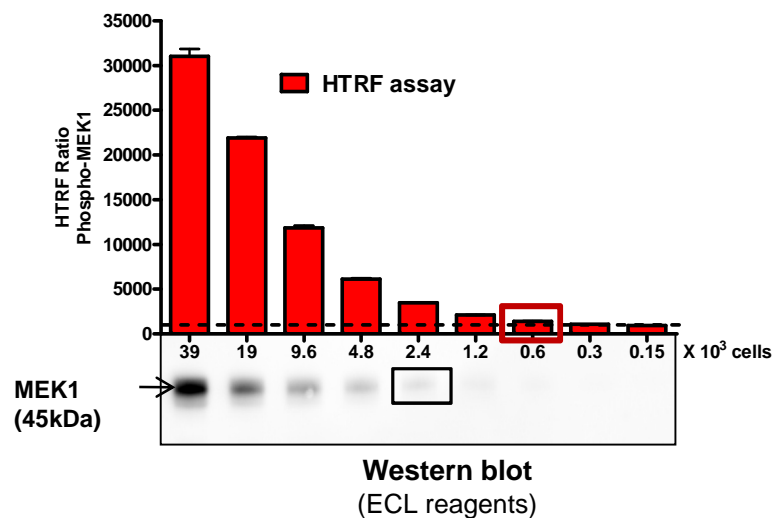


# HTRF assays are fast & convenient compare to other techniques



# HTRF® & WB are well correlated, but HTRF® often displays a superior sensitivity

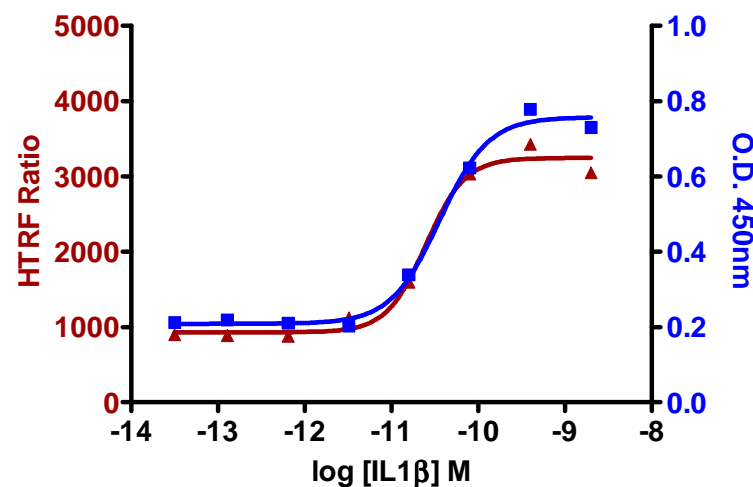
## Phospho-MEK1 (Hela cells)



Sensitivity comparison (cells / well)		
Cellular kinase	HTRF	Western-blot
P-ERK	200	1960
P-AKT	195	3125
P-Stat3	750	750
P-p38 MAPK	1250	10000
P-IKKb	580	46500
P-mTOR	1250	1250
P-CREB	6250	6250
P-MEK1	600	2400
P-MEK1/2	1200	1200
P-JNK	3120	3120
P-c Jun	2500	20000

## HTRF<sup>®</sup> & ELISA show similar results & sensitivity

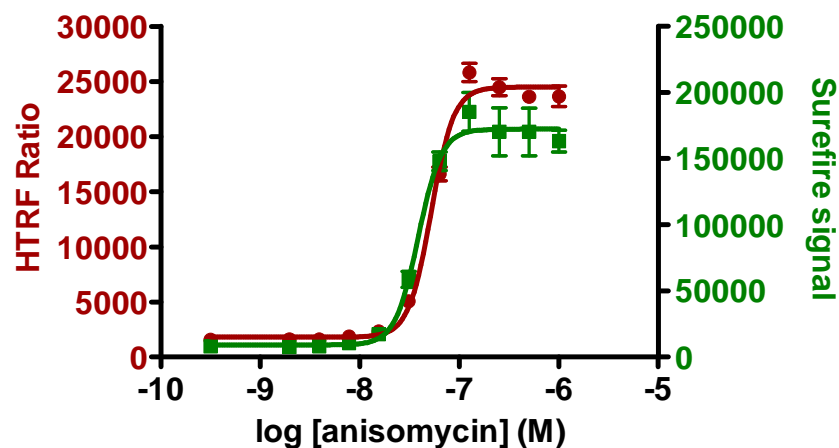
Phospho-IKKb (Hela cells)



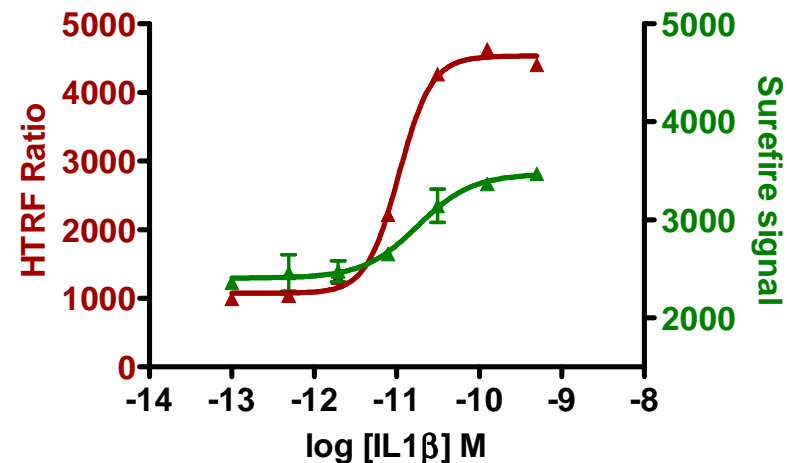
Experimental conditions			Assay Window		Compound EC50 (M)	
Cellular kinase assay	Cell line	Compound	HTRF	ELISA	HTRF	ELISA
P-Stat3 (Y705)	Hela	IFNa	7.7	5.3	10.7 <sup>E-10</sup>	5.9 <sup>E-10</sup>
P-p38 MAPK (T180-Y182)	Hela	anisomycin	10.8	3.5	7.6 <sup>E-8</sup>	8.7 <sup>E-8</sup>
P-IKKb (Ser177/181)	Hela	IL1b	3.5	3.6	2.48 <sup>E-11</sup>	3.58 <sup>E-11</sup>
P-mTOR (S2448)	HEK293	rapamycin	2.4	2.2	5.5 <sup>E-6</sup>	4.7 <sup>E-6</sup>
P-CREB (S133)	HEK293	Forskolin	4.1	4.6	1.4 <sup>E-6</sup>	1.1 <sup>E-6</sup>
P-MEK1 (S217/S222)	Hela	EGF	3.4	2.8	7 <sup>E-10</sup>	1 <sup>E-9</sup>

# HTRF<sup>®</sup> is the most sensitive homogeneous technique to monitor protein phosphorylation

Phospho-p38 (Hela cells)



Phospho-IKKb (Hela cells)

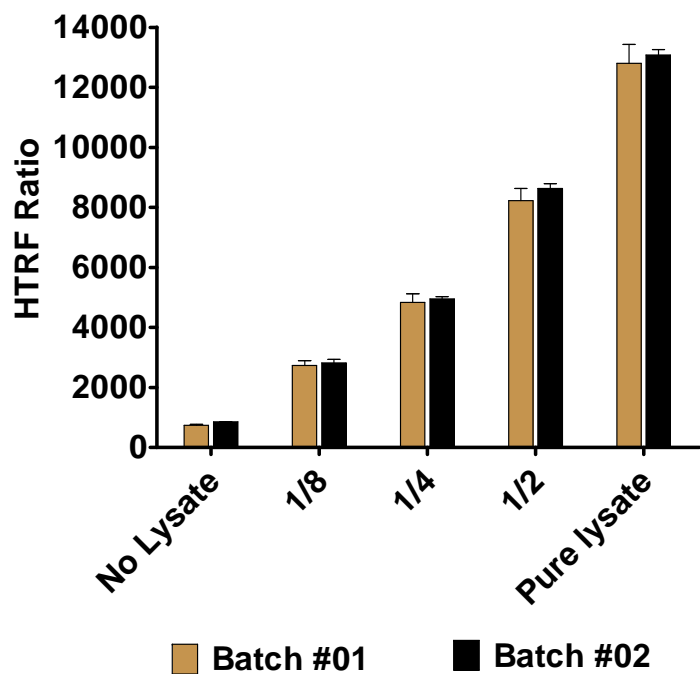


Experimental conditions			Assay Window		Compound EC50 (M)	
Cellular kinase assay	Cell line	Compound	HTRF	Surefire <sup>®</sup>	HTRF	Surefire <sup>®</sup>
P-Stat3 (Y705)	Hela	IFNa	7.7	3	10.7E <sup>-10</sup>	1.3E <sup>-9</sup>
P-p38 MAPK (T180-Y182)	Hela	anisomycin	10.8	13.5	7.6E <sup>-8</sup>	6.0E <sup>-8</sup>
P-IKKb (Ser177/181)	Hela	IL1b	3.5	1.4	2.48E <sup>-11</sup>	1.79E <sup>-11</sup>
P-mTOR (S2448)	HEK293	rapamycin	2.4	1	5.5E <sup>-6</sup>	-
P-CREB (S133)	HEK293	Forskolin	4.1	2.5	1.4E <sup>-6</sup>	3.6E <sup>-9</sup>



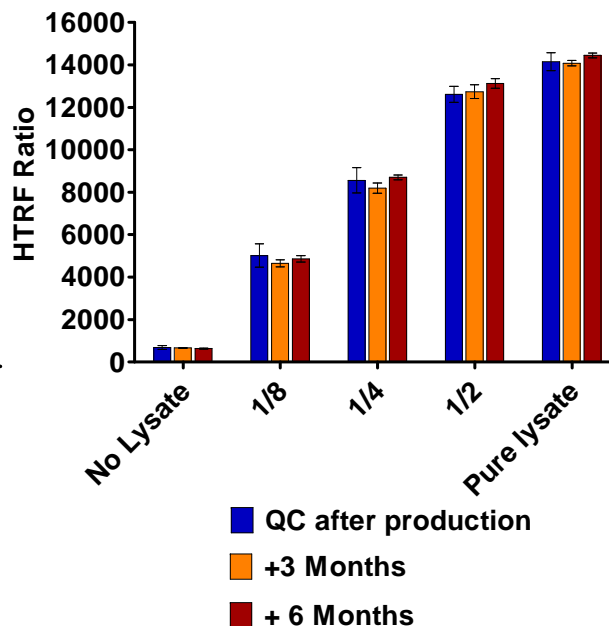
# High reagents quality enables long-term programs

**Batch to batch reproducibility  
(Phospho-P38 – HeLa cells)**

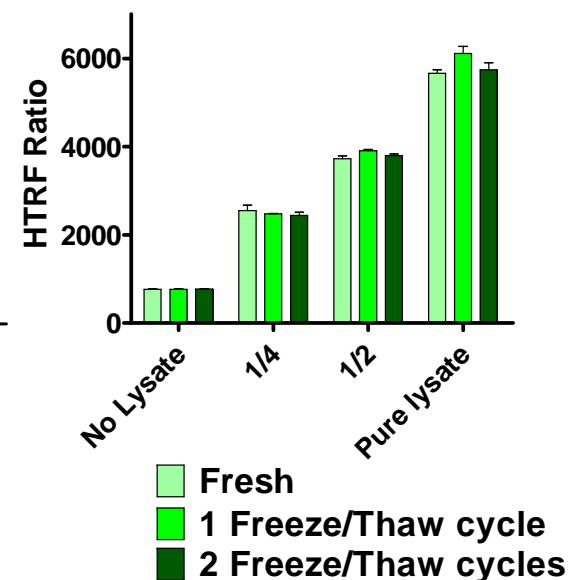


**Reagent stability**

**Long term stability  
(Phospho-mTor – HEK293)**

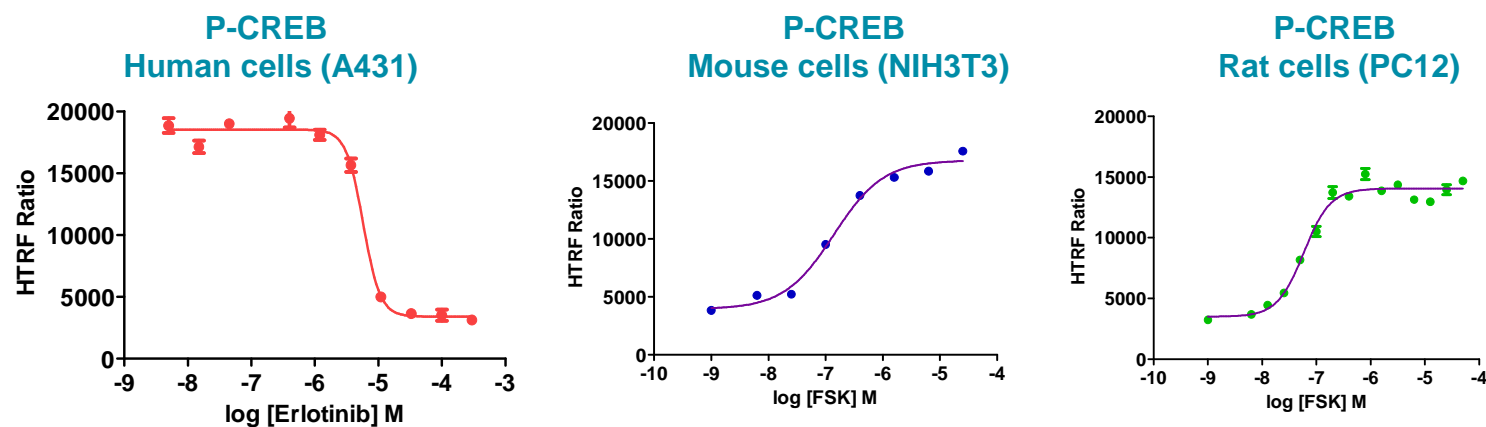


**Freeze/Thaw tolerance  
(Phospho-IKKb – HeLa cells)**

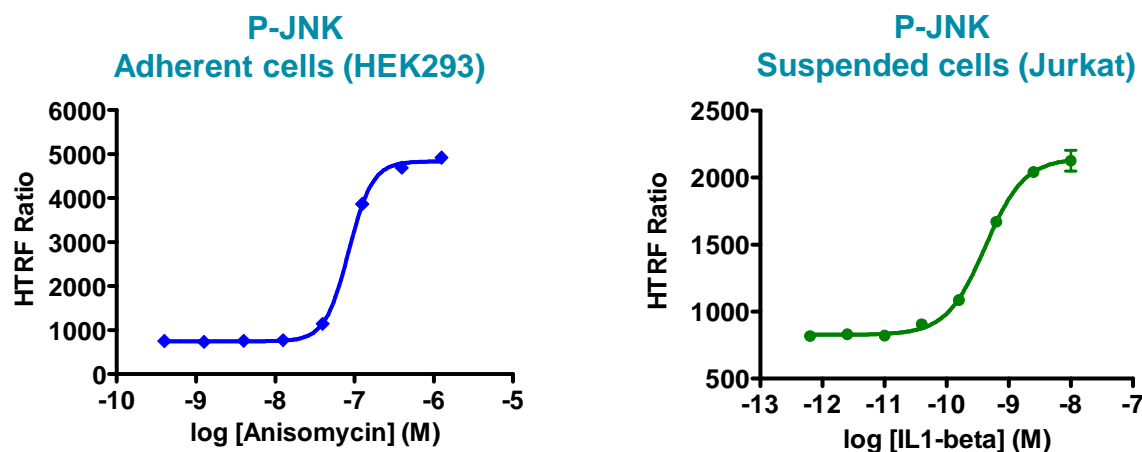


## Cell background compatibility

- HTRF<sup>®</sup> assays are usually compatible with human, mouse & rats cell lines

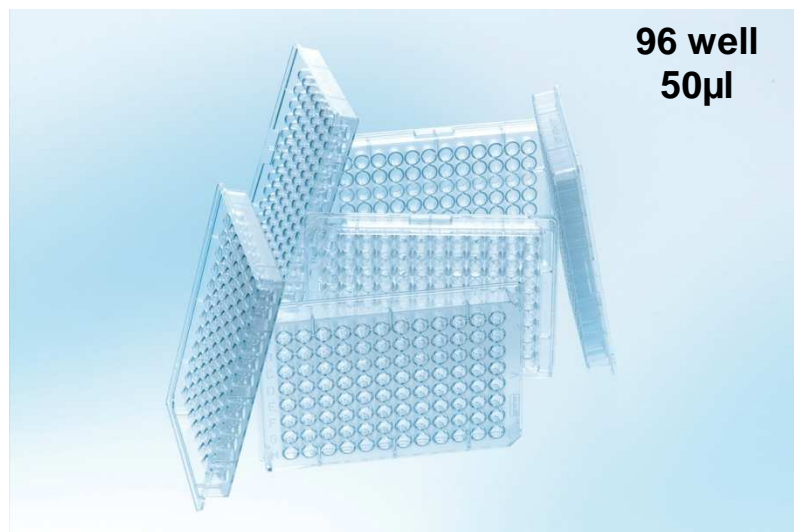


- HTRF<sup>®</sup> assays can work either with adherent or suspended cells

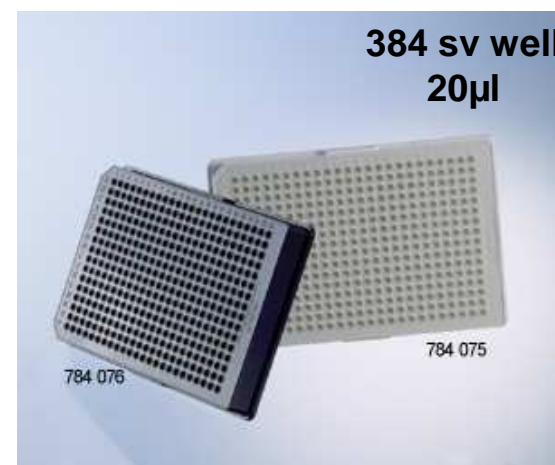


## Flexibility in assays format

**Phosphorylation Step**  
Volume of cell lysates

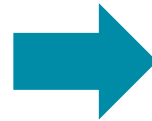
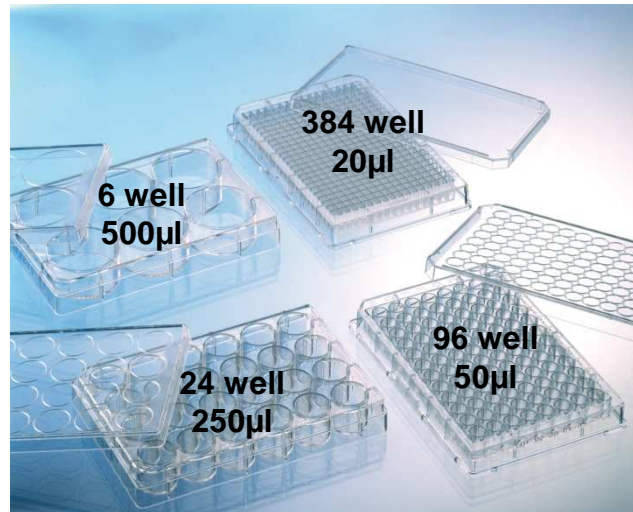
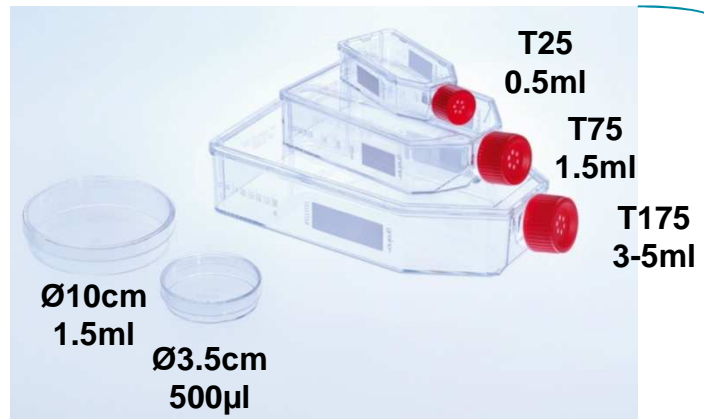


**Detection Step**  
Volume of detection

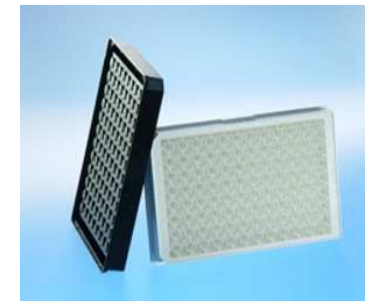


# Flexibility in assays format

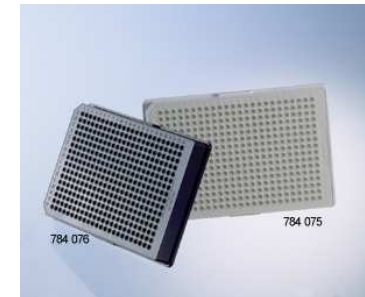
## Phosphorylation Step Volume of cell lysates



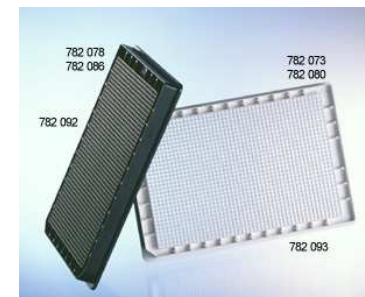
## Detection Step Volume of detection



96 Half well  
40µl



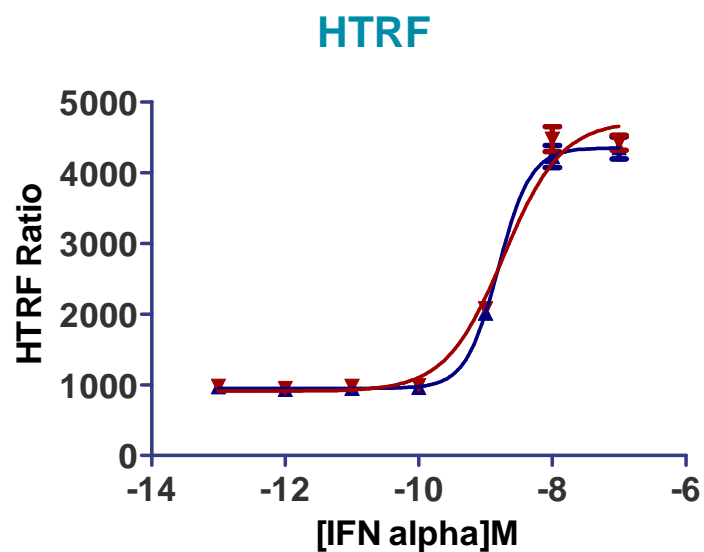
384 sv well  
20µl



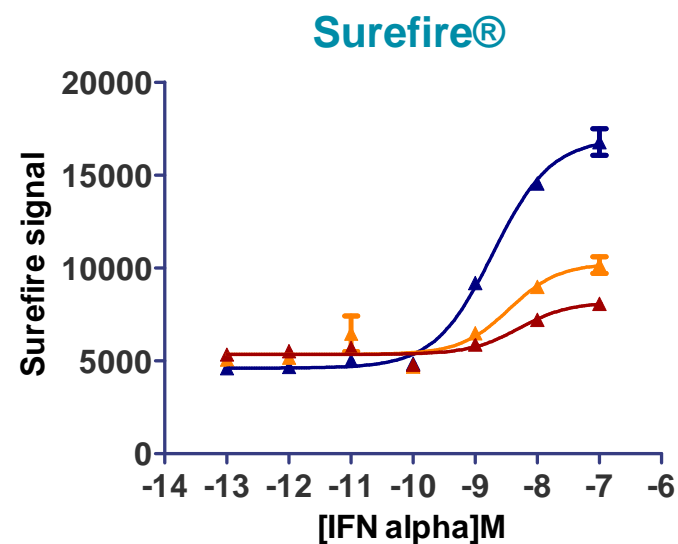
1536 well  
5-10µl

# Assay robustness

P-STAT3 assay on HeLa Cells



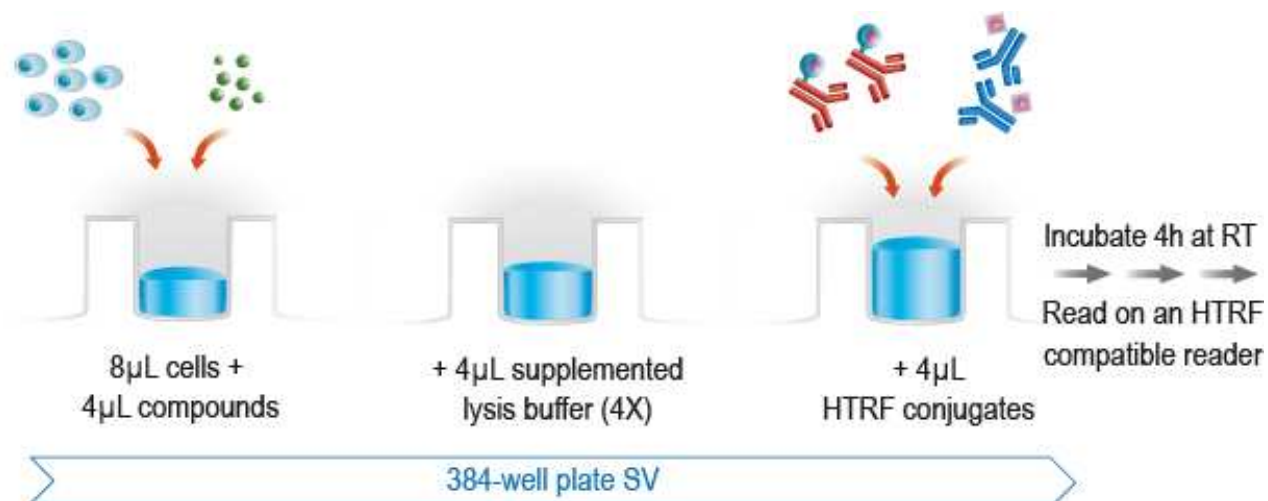
- ▲ Assay equilibrium
- ▼ Assay equilibrium + 20H



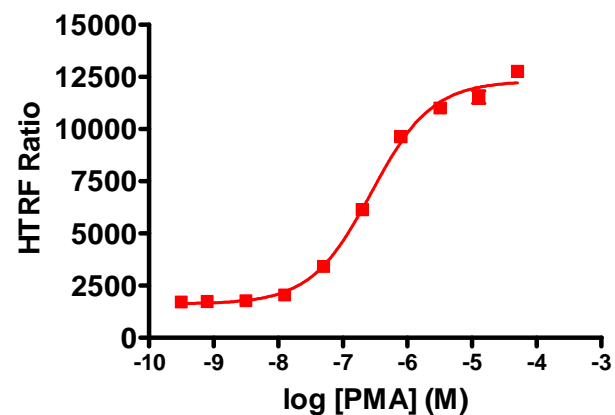
- ▲ Assay equilibrium
- ▲ Assay equilibrium + 4H
- ▲ Assay equilibrium + 20H

- HTRF<sup>®</sup> signal shows no degradation over time
- HTRF<sup>®</sup> allows multiple readouts

## HTRF<sup>®</sup> assays are amenable to HTS



### Phospho-MEK1 Jurkat Cells – 20kcells/well

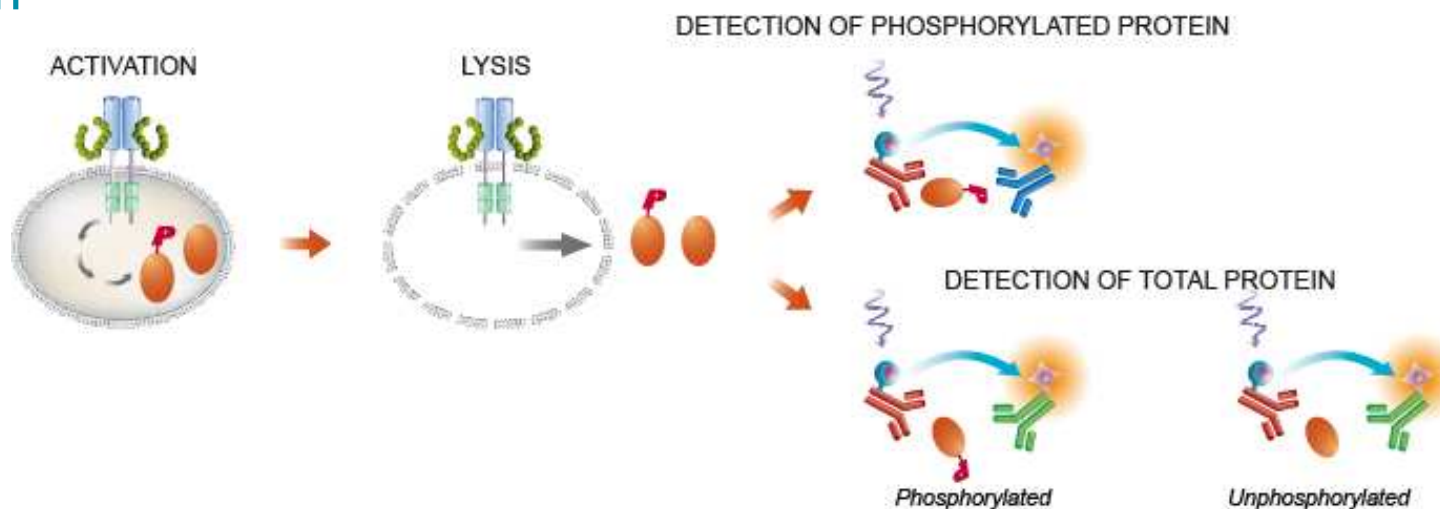


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## HTRF® Phospho assay main feature allow their use under a large variety of formats for drug research

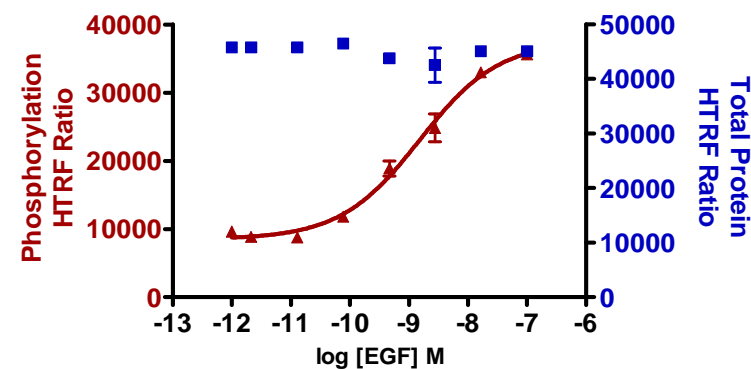
- Flexible use of HTRF kits due to high reagent quality
- Cell background compatibility
- Flexibility in assays format
- Assay robustness
- HTS amenable

## Total protein detection is now possible with the same platform



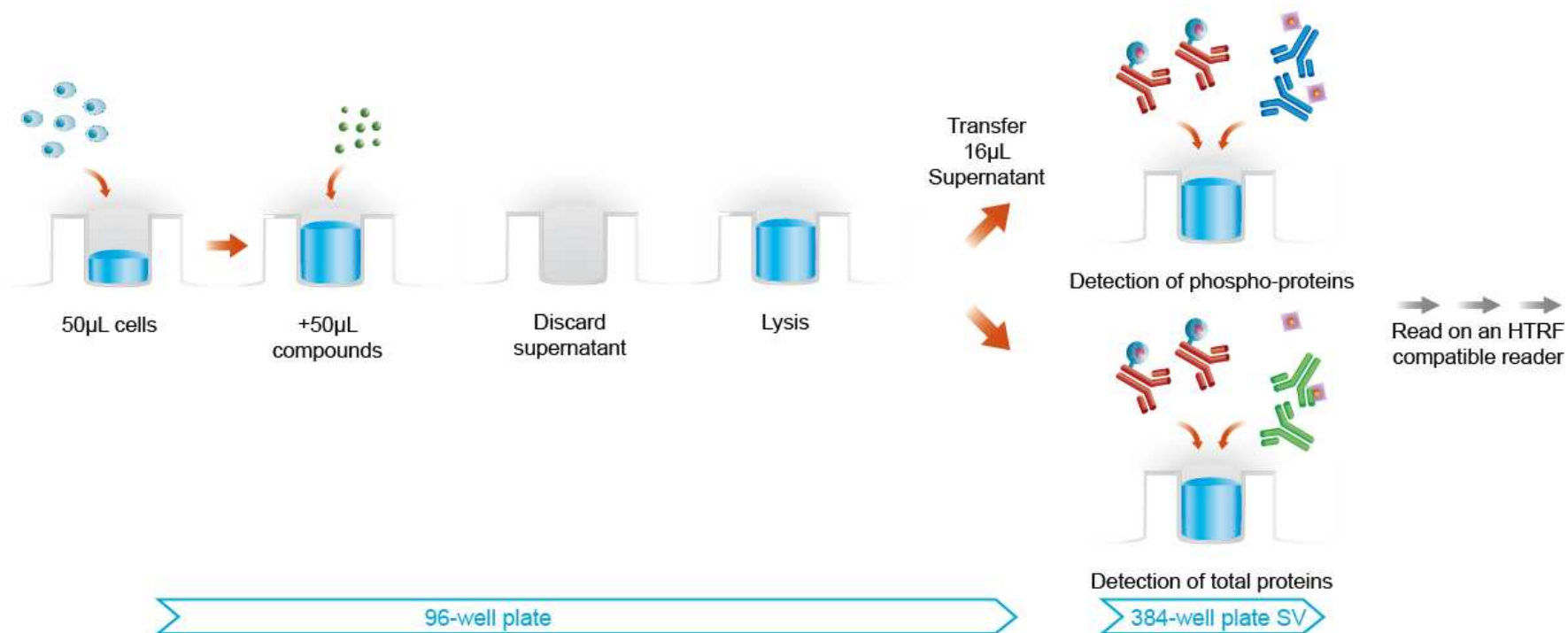
- Accurate determination of compound MOA
  - Assessment of protein expression
  - Assessment of protein phosphorylation

### Phospho & Total ERK A431 cells – 5' EGF

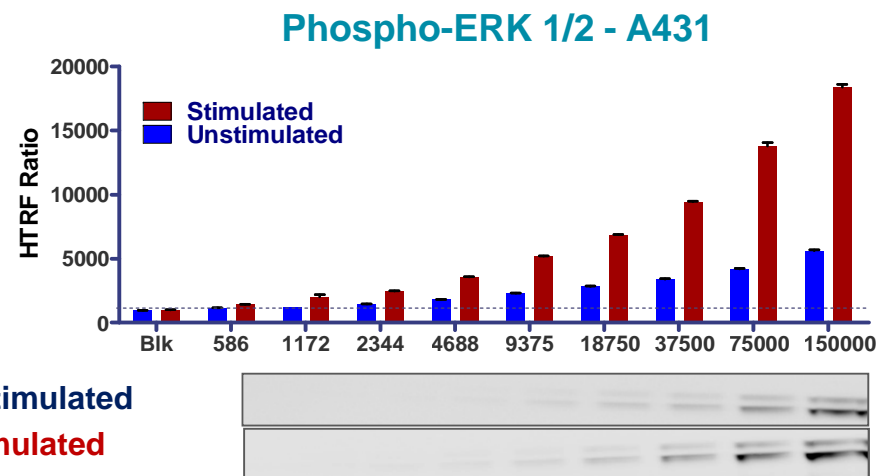
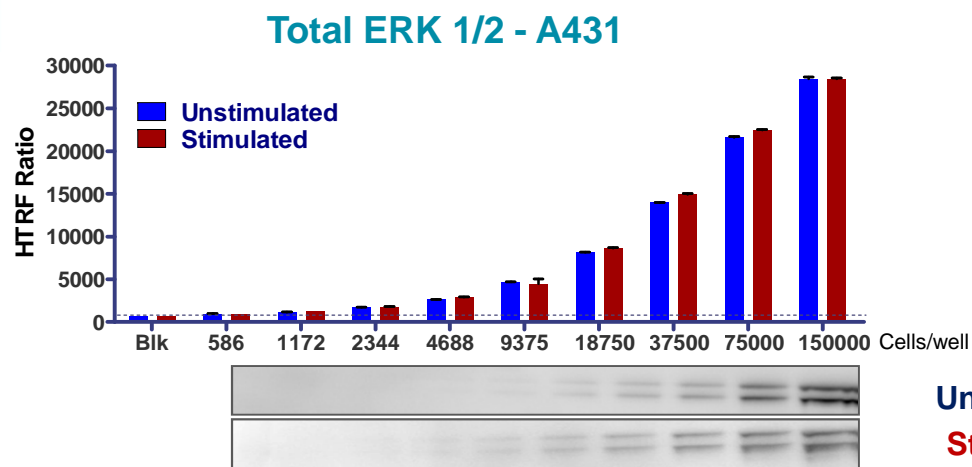




## Phospho & Total protein detection can be done with the same cell lysate



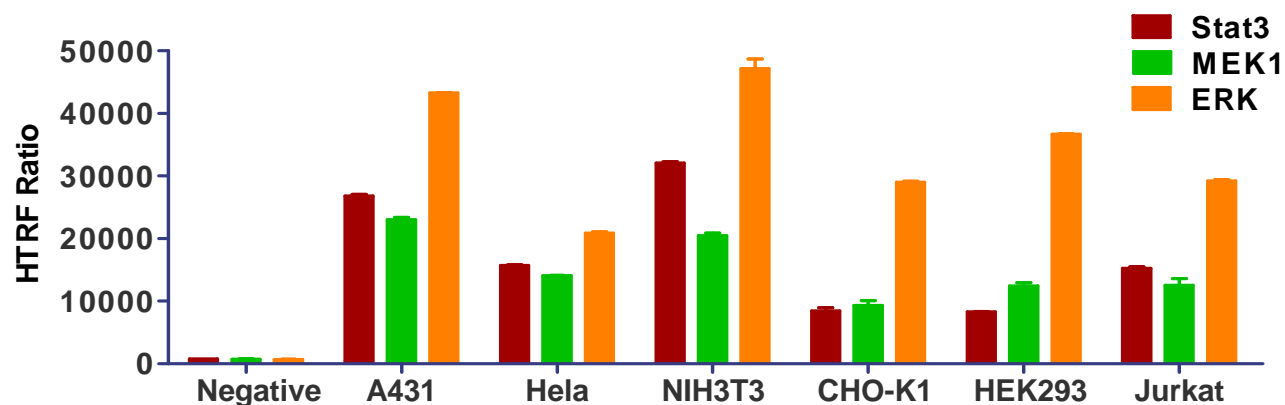
# HTRF<sup>®</sup> is as sensitive as WB to monitor total protein level



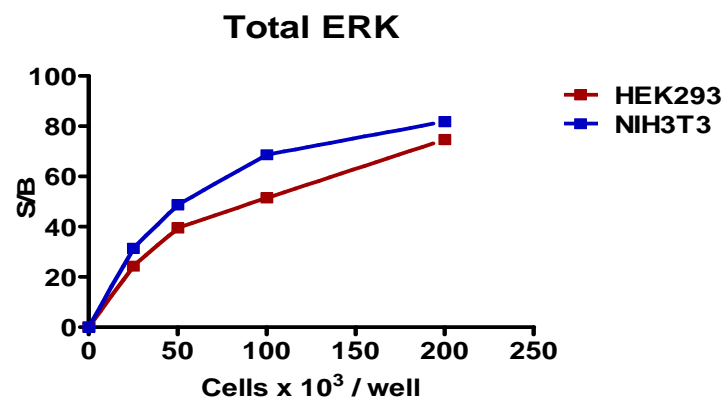
Target	Sensitivity comparison (Number of cells)	
	HTRF	WB
Stat3	1172	1172
MEK1	9375	4688
ERK 1/2	1172	4688

## Total proteins can be quantitatively detected in several species

- Validated on human, mouse, rat and hamster cell lines



- Quantitative protein detection

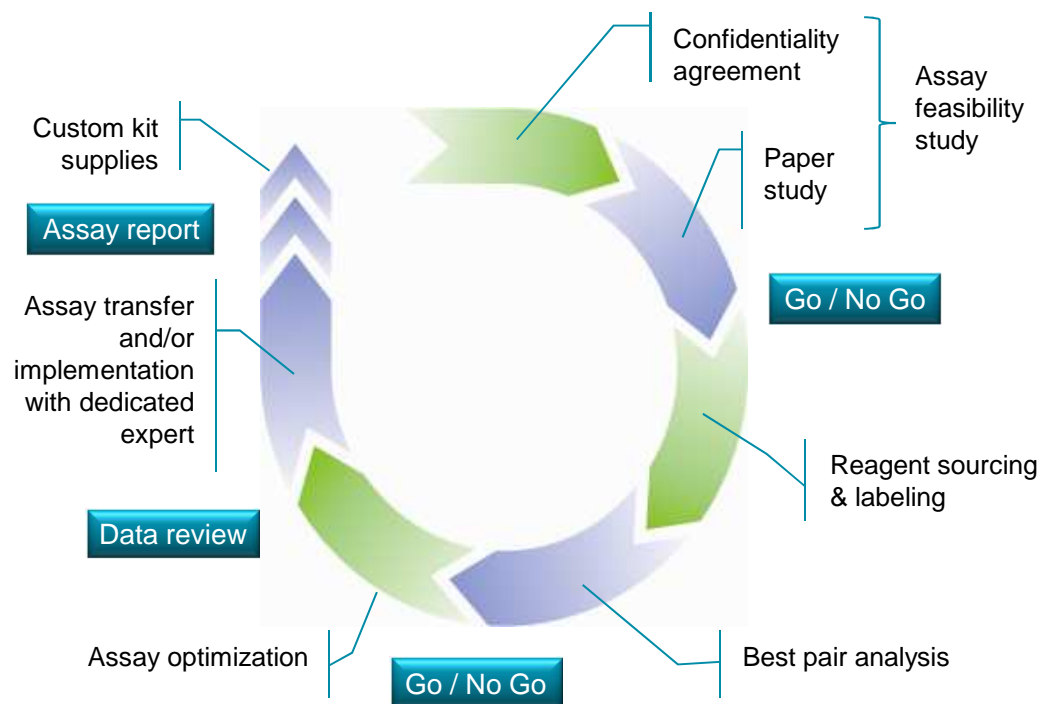


## HTRF<sup>®</sup> assay are available for a large set of targets

Phospho-protein	Total protein
Phospho-Erk1/2 (Cellul'erk)	<b>Total ERK1/2</b> <i>New</i>
Phospho-AKT (Ser473)	
Phospho-AKT (Thr308)	
<b>Phospho-JNK (Thr183/Tyr185)</b> <i>New</i>	
<b>Phospho-c Jun (Ser63)</b> <i>New</i>	
Phospho-STAT3 (Tyr705)	<b>Total-STAT3</b> <i>New</i>
Phospho-p38 MAPK (Thr180/Tyr182)	
Phospho-IKKb (Ser177/181)	
Phospho-mTOR (Ser2448)	
Phospho-CREB (Ser133)	
Phospho-EGFR	
Phospho-MEK1 (Ser218/222)	<b>Total-MEK1</b> <i>New</i>
Phospho-MEK1/2 (Ser218/222)	

- Mix & Read solutions
- Faster & equally or more sensitive than other techniques
- Cost efficient
- Amenable to all steps of drug research

## Cisbio's experts can design a custom assay for your target of interest



- **Fast assay development**
- **More than 25 custom assays developed in 2012**
- **Success rate in 2012: 90%**



Thank you