REACTION BIOLOGY

*Technology Spotlight and Applications*

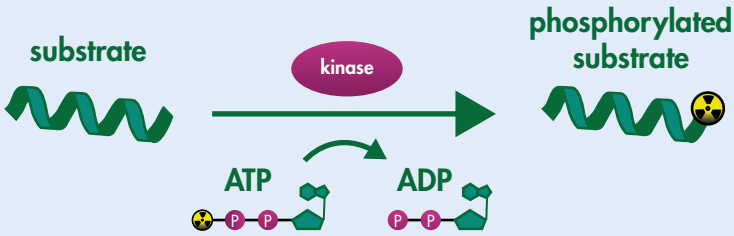
# Radiometric Filter Binding Assay

The gold standard for kinase profiling just got better

## Background Info

The radiometric filter binding assay is **the gold standard method** to study the activity of your target kinase by directly measuring its phosphorylated substrate products, without the use of modified substrates, coupling enzymes, or detection antibodies.

Radiometric assays are not kinase dependent and **can be applied to any kinase inhibitor** thus representing a truly universal assay that offers **consistent and robust results** with **low background signal.**




***1mM ATP now available on 340 wild type kinase targets***

Reaction Biology's radiometric assay protocols are further optimised to deliver best-in-class quality data, minimise error rates and avoid false positive and negatives caused by other assay formats.

Our industry-leading radiometric assay can be run using **physiologically relevant** concentrations of ATP (1mM) in addition to previously available concentrations of 1µM, 10µM, or apparent ATP-Km up to 100µM.

## Why Choose a Radiometric Assay

| | *Measures Kinase Activity* | *Detects all types of inhibitors, including substrate-specific inhibitors* | *Accommodates both peptide and protein substrates* | *No modified substrates/Additional detection reagents* | *Universally applicable to all kinases* |
|---|---|---|---|---|---|
| **Radiometric Filter Binding Assay** | ✓ | ✓ | ✓ | ✓ | ✓ |
| **FRET Peptide** | ✓ | ✓ | ✗ | ✗ | ✓ |
| **Luminescence** | ✓ | ✓ | ✓ | ✗ | ✓ |
| **Mobility Shift** | ✓ | ✓ | ✗ | ✗ | ✗ |
| **Competition Binding** | ✗ | **Suboptimal** | ✗ | ✗ | ✗ |

# Why 1mM ATP?

## Relevant and meaningful results on cellular signaling and regulatory mechanisms

Selecting 1mM ATP concentration in radiometric kinase assays can provide a more accurate representation of physiological conditions, ensuring that the obtained results are more relevant and meaningful for understanding your compound's kinase inhibitory activity.

## Here's why our 1mM ATP kinase screening solution stands out:

1. **True-to-Life Conditions:** our assays mimic typical intracellular ATP concentrations in mammalian cells, which are 1mM or higher
2. **Maximize Your Kinase Inhibitor Potential:** our conditions guarantee an accurate estimation of your target's kinase inhibitory activity and kinetics.
3. **Stability Guaranteed:** physiological ATP concentrations are required to maintain some kinase's stability, leading to accurate assay results.
4. **Relevance to Cell and *In Vivo* Data:** obtain quick biochemical data applicable to follow up cell-based testing and *in vivo* studies

# Our Solution

## HotSpot™ Kinase Screening

***"The New Gold Standard, Only at Reaction Biology"***

***Gain an in-depth understanding of your compound's specific activity and selectivity***

The largest commercially available human kinase panel of over **700 targets**

Using a proprietary radiometric assay optimised for maximal **sensitivity** and **accuracy**

Now available at physiologically relevant **1mM ATP** for 340 *wild type* kinases

# Discover the Benefits

### Flexible

**Choose any screening design**

- $IC_{50}$ value determination with 5 or 10 concentrations
- 1 or 2 doses per compound

### Fast

- Results in as fast as 10 business days for standard projects
- Run bi-weekly

### Quality data

- A kinase-specific control compound included for each in 10-dose $IC_{50}$

### Physiologically relevant

- Now available using 1mM $^{33}$P-γ-ATP on 340 *wild type* kinase targets

# ATP-Max KinomeScreen

A full panel of 340 kinase targets available at 1mM ATP, for comprehensive and deep interrogation of your compound's selectivity against the whole human kinome
Available as a full panel, or select targets to create your own custom panel.

| Kinase Family | ATP-MAX Kinome Targets |
|---|---|
| AG | MSK2/RPS6KA4 |
| AGC | AKT1, AKT2, AKT3, DMPK2, GRK2, GRK3, GRK4, GRK5, GRK6, GRK7, LATS1, LATS2, LYN-B, MAST3, MASTL, MRCKα/CDC42BPA, MRCKb/CDC42BPB, MSK1/RPS6KA5, P70S6K/RPS6KB1, P70S6KB/RPS6KB2, PDK1/PDPK1, PKA, PKACB, PKACG, PKCα, PKCβ1, PKCβ2, PKCδ, PKCε, PKCη, PKCγ, PKCι, PKCθ, PKCζ, PKG1α, PKG1b, PKG2/PRKG2, PKN1/PRK1, STK32B/YANK2, PRKX, ROCK1, ROCK2, RSK1, RSK2, RSK3, RSK4, SGK1, SGK2, SGK3/SGKL, STK21/CIT, STK38/NDR1, STK38L/NDR2 |
| CAMK | ARK5/NUAK1, BRSK1, BRSK2, CAMK1A, CAMK1B, CAMK1D, CAMK1g, CAMK2α, CAMK2b, CAMK2D, CAMK2G, CAMK4, CHK1, CHK2, DAPK1, DAPK2, DCAMKL1, DCAMKL2, LKB1, MAPKAPK2, MAPKAPK3, MAPKAPK5/PRAK, MARK1, MARK2/PAR-1BA, MARK3, MARK4, MELK, MLCK/MYLK, MLCK2/MYLK2, MNK1, MNK2, MYLK3, MYLK4, NIM1, PASK, PHKG1, PHKG2, PIM1, PIM2, PIM3, PKCMU/PRKD1, PKCNU/PRKD3, PKD2/PRKD2, SIK1, SIK2, SIK3, SNARK/NUAK2, SNRK, STK22D/TSSK1, TSSK2, TSSK3/STK22C, ZIPK/DAPK3 |
| CK1 | CK1α1, CK1α1L, CK1δ, CK1ε, CK1γ1, CK1γ2, CK1γ3, TTBK1, TTBK2, VRK2 |
| CMGC | CDK1/CYCLIN A, CDK1/CYCLIN B, CDK1/CYCLIN E, CDK14/CYCLIN Y (PFTK1), CDK15/CYCLIN A2, CDK15/CYCLIN B1, CDK16/CYCLIN Y (PCTAIRE), CDK17/CYCLIN Y (PCTK2), CDK18/CYCLIN Y (PCTK3), CDK2/CYCLIN A, CDK2/CYCLIN A1, CDK2/CYCLIN E, CDK2/CYCLIN E2, CDK2/CYCLIN O, CDK3/CYCLIN C, CDK3/CYCLIN E, CDK3/CYCLIN E2, CDK4/CYCLIN D1, CDK4/CYCLIN D2, CDK4/CYCLIN D3, CDK5/P25, CDK5/P35, CDK6/CYCLIN D1, CDK6/CYCLIN D2, CDK6/CYCLIN D3, CDK7/CYCLIN H, CDK9/CYCLIN K, CDK9/CYCLIN T1, CDK9/CYCLIN T2, CK2A, CK2A2, CLK1, CLK2, CLK3, CLK4, DYRK1/DYRK1A, DYRK1B, DYRK2, DYRK3, ERK1, ERK2/MAPK1, ERK5/MAPK7, ERK7/MAPK15, GSK3A, GSK3B, HIPK1, HIPK2, HIPK3, HIPK4, JNK1, JNK2, JNK3, LCK2/ICK, MAK, MSSK1/STK23, P38A/MAPK14, P38B/MAPK11, P38d/MAPK13, P38G, SRPK1, SRPK2 |
| STE | ASK1/MAP3K5, COT1/MAP3K8, GCK/MAP4K2, GLK/MAP4K3, HGK/MAP4K4, HPK1/MAP4K1, KHS/MAP4K5, LOK/STK10, MEK1, MEK3, MEK5, MEKK1, MEKK2, MEKK3, MINK/MINK1, MKK4, MKK6, MKK7, MST1/STK4, MST2/STK3, MST3/STK24, MYO3A, MYO3B, OSR1/OXSR1, PAK1, PAK2, PAK3, PAK4, PAK5, PAK6, SLK/STK2, STK25/YSK1, STK39/STLK3, TAOK1, TAOK2/TAO1, TAOK3/JIK, TNIK, YSK4/MAP3K19 |
| TK | ABL1, ABL2/ARG, ACK1, ALK, AXL, BLK, BMX/ETK, BRK, BTK, C-KIT, C-MER, C-MET, CSK, C-SRC, CTK/MATK, DDR2, EGFR, EPHA1, EPHA2, EPHA3, EPHA4, EPHA5, EPHA6, EPHA7, EPHA8, EPHB1, EPHB2, EPHB3, EPHB4, ERBB2/HER2, ERBB4/HER4, FAK/PTK2, FER, FES/FPS, FGFR1, FGFR2, FGFR3, FGFR4, FGR, FLT1/VEGFR1, FLT3, FLT4/VEGFR3, FMS, FRK/PTK5, FYN, HCK, IGF1R, IR, IRR/INSRR, ITK, JAK1, JAK2, JAK3, KDR/VEGFR2, LCK, LYN, MUSK, PDGFRA, PDGFRB, PYK2, RET, RON/MST1R, ROS/ROS1, SRMS, SYK, TEC, TIE2/TEK, TRKA, TRKB, TRKC, TXK, TYK1/LTK, TYK2, TYRO3/SKY, YES/YES1, ZAP70 |
| TKL | ALK4/ACVR1B, ALK5/TGFBR1, ALK6/BMPR1B, ARAF, BMPR2, BRAF, IRAK1, IRAK4, LIMK1, LIMK2, LRRK2, MLK1/MAP3K9, MLK2/MAP3K10, MLK3/MAP3K11, MLK4, PBK/TOPK, RAF1, RIPK2, RIPK4, RIPK5, TESK1, TESK2, TGFβR2, ZAK/MLTK |
| Other | AUR-A, AUR-B, AUR-C, CAMKK2, ERN1/IRE1, ERN2/IRE2, HASPIN, IKKβ/IKBKB, IKKε/IKBKE, NEK1, NEK2, NEK3, NEK4, NEK5, NEK6, NEK7, NEK9, PLK1, PLK2, PLK3, TBK1, TLK1, TLK2, ULK1, ULK2, ULK3, WNK3 |

# ATP-Max Diversify

A tailored panel of representative isoforms from each major kinase family available at 1mM ATP, for quick understanding of your compound's selectivity

| Kinase Family | ATP-Max Diversify targets |
|---|---|
| **AGC** | MAST3, ROCK1, PKAα, PKCα, PKCθ, Akt1/PKBα, RSK1, p70S6K, MSK2 |
| **CAMK** | caMLCK, DAPK2, TSSK1, Pim3, Chk1, ARK5, MARK2, MNK2, CAMK1α, DCAMKL1 |
| **CK1** | CK1α, CK2α, CK1γ1 |
| **CMGC** | ERK1, p38α, CDK4, JNK3, CDK7, CDK9, CDK1, GSK3β, CLK2, HIPK2, DYRK1A |
| **TKL** | Mlk1, ALK4, BRAF, TGFβR2, LIMk1, IRAK4 |
| **TK** | Abl1, BTK, TEC, Lyn, Src, EphAS, EphB2, IGF1R, TrkA, Met, Ret, FGFR2, FLT1/VEGFR1, Kit, FLT3, EGFR, Jak2 |
| **STE** | MST1, LOK, PAK4, MEK1/MAP2K1, MEKK1 |
| **Lipid Kinases** | PI3Kα, PI3Kβ, PI3Kδ, PI3Kγ |
| **Atypical Kinases** | AurA, Nek1, ULK1, IKKε, PLK1 |

Discuss your project directly with our kinase experts

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