

Supplementary Table 1. **Qualitative and quantitative operational checkpoints in the μ -ivkra assay**

Step	Acquisition time	Purpose	Monitored area
After loading kinase reaction buffer	1 min	<ul style="list-style-type: none"> • Verify equal loading • Verify proper chip operation 	Circulation chamber & upper bead columns
After the kinase reaction	1 min	<ul style="list-style-type: none"> • Verify homogenous mixing • Verify proper chip operation 	Circulation chamber & upper bead columns
After pumping the reaction solution to the lower columns	10 min	<ul style="list-style-type: none"> • Monitor flow • Check radioactivity background • Measure the degree of kinase autophosphorylation 	Circulation chamber & upper bead columns
After washing the lower streptavidin columns	20 min	<ul style="list-style-type: none"> • Verify substrate capture • Quantify the kinase activity via incorporated radioisotope 	Lower bead trapping columns

Summary of key checkpoints during a μ -ivkra assay. Four checkpoints at different steps in the procedure are used to confirm a successful assay and verify proper physical and operational performance of the device.

Supplementary Table 2. **Relative reaction efficiency between on-chip and off-chip kinase assays**

$$\text{kinase assay efficiency} = \frac{\text{pCi incorporated} / \text{specific activity}}{\text{lysate volume} \times \text{reaction time}}$$

	<u>Off-chip</u>	<u>On-chip</u>
^{32}P incorporated (pCi) *	103616	2797
relative specific activity (relative hot ATP/cold ATP) †	1	33
lysate volume (uL)	400	0.05
reaction time (min)	30	15
efficiency (arbitrary units), normalizing for the factors listed above	12.3	113
relative efficiency, normalizing for the factors listed above	1	13.1
reaction velocity/ V_{\max} ‡	0.78	0.48
relative efficiency, also normalizing for reaction velocity	1	21.6

* For the off-chip assay only 1/30th of the reaction products were measured by liquid scintillation and the result reported is the readout multiplied by 30. For the on-chip assay only one half of the reaction products were captured by the streptavidin columns (see Supplementary Fig. 5), and the result reported is the beta camera readout multiplied by 2. Results reported are the average of 3 representative experiments. † The on-chip reaction uses a 33-fold higher hot to cold ATP ratio than the off-chip reaction (see Methods). ‡ Calculated using the Michaelis-Menten equation $Velocity = (V_{\max} \times [substrate]) / (K_m + [substrate])$, the K_m for BCR-ABL p210 and Abltide of 27.6 μM (1) and a peptide substrate concentration of 100 μM for the off-chip reaction and 25 μM for the on-chip reaction.

1. Skaggs BJ, Gorre ME, Ryvkin A, Burgess MR, Xie Y, Han Y, Komisopoulou E, Brown LM, Loo JA, Landaw EM, Sawyers CL, Graeber TG. Phosphorylation of the ATP-binding loop directs oncogenicity of drug-resistant BCR-ABL mutants. Proc Natl Acad Sci U S A 2006; 103: 19466-71.