# FlashPlate-based Protein Kinase Assay Protocol ($^{33}$PanQinase® Assay)

## Standard Procedure for all kinases (Manual Assay)

### Materials:

| | |
|---|---|
| **ATP** | (e.g. Sigma, # A-7699) |
| **$CaCl_2$** | (e.g. VWR, # 1.02382) |
| **Calmodulin** | (e.g. Millipore, # 14-368) |
| **cGMP** | (e.g. Sigma, # G-6129) |
| **1,2-Dioleoyl-glycerol** | (e.g. Sigma, # D-8394) |
| **DNA** | (e.g. Sigma, # D-4522) |
| **DMSO** | (e.g. Sigma, # 154938) |
| **DTT** | (e.g. Sigma, # D-0632) |
| **EDTA** | (e.g. Roth, # 8040.1) |
| **$H_2O$ p.a.** | (e.g. VWR, # 1.16754.9010) |
| **HEPES** | (e.g. Sigma, # H-3375) |
| **$MgCl_2$** | (e.g. Sigma, # M-3634) |
| **$MnCl_2$** | (e.g. VWR, # 1.05927.1000) |
| **NaCl** | (e.g. Merck, # 1.06404) |
| **Na-orthovanadate** | (e.g. Sigma, # S-6508) |
| **Ortho-phosphoric acid** | (e.g. VWR, # 1.00563.1000) |
| **$PEG_{20.000}$** | (e.g. SERVA, # 33138) |
| **Phosphatidyl-serine** | (e.g. Fluka, # 79405) |
| (10 mg/ml in chloroform/methanol) | |
| **$^{33}$P-γ-ATP** | (PerkinElmer, # NEG 302 H) |
| **96-well MTP-FlashPlate**, (Polystyrol) | (PerkinElmer, # SMP 200) |
| **384-well MTP-FlashPlate**, (Polystyrol) | (PerkinElmer, # SMP400) |

### Final Assay Conc. (50 µl reaction cocktail):

- 70 mM HEPES-NaOH, pH 7.5
- 3 mM $MgCl_2$
- 3 mM $MnCl_2$
- 3 µM Na-orthovanadate
- 1.2 mM DTT
- 50 µg/ml $PEG_{20.000}$
- ATP (variable, corresponding to the app. ATP-$K_m$ of the respective kinase
- [γ–$^{33}$P]-ATP: approx. 7-8 x $10^5$ cpm
- 1 % (v/v) DMSO
- Substrate (variable)
- Recombinant Protein Kinase (variable)

All PKC assays (except the PKC-mu and the PKC-nu assay) additionally contain 1 mM $CaCl_2$, 4 mM EDTA, 5 µg/ml Phosphatidylserine and 1 µg/ml 1,2-Dioleyl-glycerol.

The CAMK1D, CAMK2A, CAMK2B, CAMK2D, CAMK4, CAMKK1, CAMKK2, DAPK2, EEF2K, MYLK, MYLK2 and MYLK3 assays additionally contain 1 µg/ml Calmodulin and 0.5 mM $CaCl_2$.

The PRKG1 and PRKG2 assays additionally contain 1 µM cGMP.

The DNA-PK assay additionally contains 2.5 µg/ml DNA.

### Prepare in advance (store at 4° C):

**Standard-Assay-Buffer (2.5x stock)**

- 125 mM HEPES-NaOH, pH 7.5
- 7.5 mM $MgCl_2$
- 7.5 mM $MnCl_2$
- 7.5 µM Na-orthovanadate
- 2.5 mM DTT

**Kinase-Dilution-Buffer (10x stock)**

- 500 mM HEPES-NaOH, pH 7.5
- 2.5 mg/ml $PEG_{20.000}$
- 10 mM DTT

### Manual Assay Procedure:

**In FlashPlate:**

1. Add 20 µl Standard-Assay-Buffer (2.5x stock)
2. Add 5 µl 10% DMSO *(w, w/o test cpd)*
3. Add 10 µl substrate (in 50mM HEPES pH 7.5)
4. Add 10 µl recombinant protein kinase (in 1x Kinase-Dilution-Buffer)
5. Add 5 µl ATP (in $H_2O$)
6. Mix on shaker
7. Incubate for 60 min at 30°C
8. Stop reaction with 50 µl 2% $H_3PO_4$
9. Mix on shaker
10. Wash 3 times with 200 µl 0.9 % NaCl
11. Count dry plate with Scintillation Counter