

Europium-anti-phospho-PLK (Ser137) Antibody

Product Numbers:
TRF0203-D (10 µg, 1,562 assay points)
TRF0203-M (100 µg, 15,625 assay points)

SPECIFIC LOT DATA

LOT NUMBER:	XXXXX
DESCRIPTION:	Europium-labeled rabbit polyclonal antibody recognizing phospho-Ser137 in human polo-like kinase (PLK)
FORMAT:	1,562 assay points (TRF0203-D) or 15,625 assay points (TRF0203-M) (assuming 40 fmol/assay point)
AMOUNT:	10 µg (TRF0203-D) or 100 µg (TRF0203-M)
CONCENTRATION:	100 µg/mL (0.625 µM)
LABELING REAGENT:	Eu-W1024 chelate
LABELING YIELD:	<input checked="" type="checkbox"/> Europium chelate/antibody
PACKAGING:	50 mM Tris-HCl pH 7.4, 0.9% NaCl, 0.1% BSA and 0.05% sodium azide as preservative
STORAGE:	Store at +4°C
RECOMMENDATION:	Spin down briefly before use to improve recovery of content
EXPIRY DATE:	Month, day, year

KINASE ASSAY APPLICATION

AURORA A KINASE: ATP TITRATION

MATERIALS:

Substrate:	ULight [™] -PLK (Ser137) Peptide	PerkinElmer # TRF0110
Antibody:	Europium-anti-phospho-PLK (Ser137)	PerkinElmer # TRF0203
Kinase:	Aurora A, active	Upstate # 14-511
Detection Buffer:	LANCE [®] Detection Buffer, 10X	PerkinElmer # CR97-100
Plate:	OptiPlate [™] -384, white	PerkinElmer # 6007299
TopSeal [™] :	TopSeal-A	PerkinElmer # 6005185

SUGGESTED METHOD:

(Specific applications might require optimization)

Reagent Preparation

- Prepare 1X Kinase Assay Buffer: 50 mM HEPES pH 7.5, 1 mM EGTA, 10 mM MgCl₂, 2 mM DTT and 0.01% Tween-20.
- Prepare a 2X Aurora A solution: dilute enzyme to a concentration of 8 nM in Kinase Assay Buffer. Keep on ice.
- Prepare a 4X ULight-PLK solution: dilute ULight-PLK to a concentration of 200 nM in Kinase Assay Buffer.
- Prepare a 4X ATP solution: dilute ATP to concentrations ranging from 40 nM to 4 mM (serial half-log dilutions) in Kinase Assay Buffer. Keep on ice.
- Prepare 1X Detection Buffer: dilute 0.5 mL of 10X Detection Buffer with 4.5 mL of H₂O.
- Prepare a 4X Stop solution*: dilute EDTA to a concentration of 40 mM in 1X Detection Buffer.
- Prepare a 4X Detection Mix: dilute Europium-anti-phospho-PLK to a concentration of 8 nM in 1X Detection Buffer.

Protocol

- Pipet 5 µL of 2X Aurora A solution into a 384-well white OptiPlate-384 (4 nM final concentration).
- Add 2.5 µL of 4X ULight-PLK solution (50 nM final concentration).
- Add 2.5 µL of 4X ATP solution (10 nM to 1 mM final concentrations).
- Cover plate with TopSeal-A and incubate 60 min at 23°C.
- Add 5 µL of 4X Stop solution* and incubate 5 min at 23°C.
- Add 5 µL of Detection Mix (2 nM Eu-anti-phospho-PLK antibody final concentration).
- Cover plate with TopSeal-A and incubate 60 min at 23°C.
- Remove TopSeal-A and read in TR-FRET mode at 665 nm (excitation at 320 or 340 nm).

*Alternatively, the Stop solution and Detection Mix can be premixed and added together to the kinase reaction.

TYPICAL ATP TITRATION DATA obtained using the EnVision[®] Multilabel Reader:

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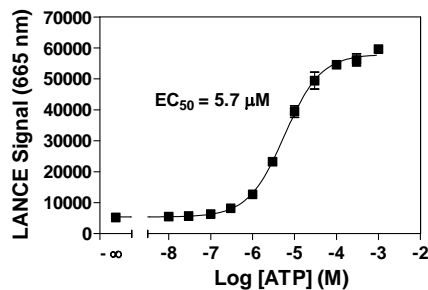
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Aurora A kinase assay using the *ULight*TM-PLK Peptide
and Eu-anti-phospho-PLK Antibody



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