

DELFLIA[®] Tyrosine Kinase kit**AD0122****For Research Use Only****INTENDED USE**

This DELFLIA[®] Tyrosine Kinase kit is intended for measuring of tyrosine kinase activity.

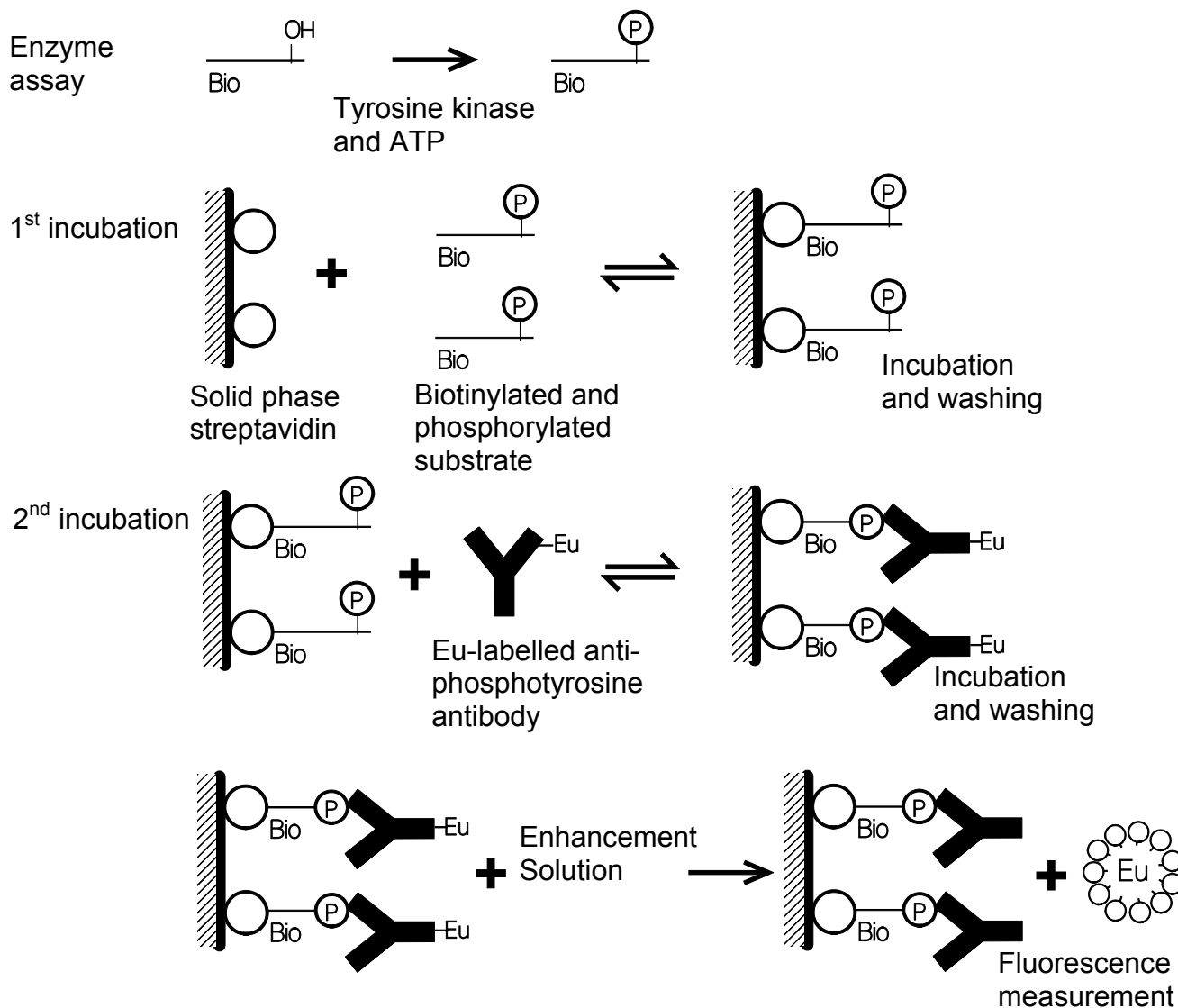
INTRODUCTION

Protein phosphorylation is a basic mechanism for the modification of protein function in eukaryotic cells. The level of phosphorylated tyrosine in many cellular proteins increases causing various activation processes which are mediated through phosphotyrosine kinases.

PRINCIPLES OF THE ASSAY

DELFLIA Tyrosine Kinase assay is a dissociation enhanced time-resolved fluorometric assay. First the enzyme assay is carried out normally with biotinylated substrate supplied with the kit, then the reaction solution is diluted and transferred to Streptavidin plate. Antibody against phosphorylated tyrosine binds to phosphorylated substrate. Positive control (biotinylated phosphopeptide) can also be used to test the assay procedure.

Enhancement Solution dissociates europium ions from the labelled anti-phosphotyrosine antibody into solution, where they form highly fluorescent chelates with components of the Enhancement Solution. The fluorescence in each well is then measured. The fluorescence of each sample is proportional to tyrosine kinase phosphorylation activity.



KIT CONTENTS

Reagents

Component	Quantity	Shelf life and storage
DELFIA Eu-N1-labelled antiphosphotyrosine antibody (PT66)	1 vial, 15 µg	+2 - +8°C until expiry date stated on the vial label.

The PT66 antibody is an affinity purified IgG1 subclass of mouse monoclonal antibody. The labelled antibody (200 µg/mL) is in Tris-HCl buffered (pH 7.8) salt solution with < 0.1 % sodium azide as preservative.

Biotinylated poly(Glu,Ala,Tyr) Substrate	1 vial, 10 nmol as precipitate	+2 - +8°C. After reconstitution store at -20°C.
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Substrate for the enzyme assay, biotinylated poly(Glu,Ala,Tyr 6:3:1) peptide.

The Biotinylated poly(Glu,Ala,Tyr) Substrate has been precipitated from 200 µL of buffer containing 20 mmol/L Tris-HCl, pH 7.5 and 50 mmol/L NaCl. The precipitated substrate should be dissolved in water containing 0.02 % Brij 35¹, e.g. in 1 mL giving 10 µmol/L solution.

The amount of substrate is enough for 200 assays, when using 0.5 µmol/L substrate concentration in the enzyme assay. The reaction solution should be diluted to 1:10 or 1: 50 in Assay Buffer before adding to Streptavidin coated plate.

Positive control	1 vial, 0.5 nmol	+2 - +8°C
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Biotinylated phosphopeptide (2500 nmol/L) in Tris-HCl buffered (pH 7.8) salt solution with 0.1 % BSA, and < 0.1 % sodium azide as preservative.

Wash Concentrate	2 bottles, 40 mL	+2 - +8°C until expiry date stated on the bottle label.
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A 25-fold concentration of Tris-HCl buffered (pH 7.8) salt solution with Tween 20. Contains Germall II² as preservative.

Assay Buffer	2 bottles, 50 mL	+2 - +8°C until expiry date stated on the bottle label.
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Ready-for-use Tris-HCl buffered (pH 7.8) salt solution with bovine serum albumin, bovine globulin, Tween 40, an inert red dye, and < 0.1 % sodium azide as preservative.

Enhancement Solution	1 bottle, 50 mL	+2 - +8°C until expiry date stated on the bottle label. Shelf life 6 months at room temperature (+20 - +25°C). Avoid direct sunlight.
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Ready-for-use Enhancement Solution with Triton X-100³, acetic acid and chelators.

Streptavidin Microtitration Strips	2 plates	+2 - +8°C until expiry date stated on the label. Make sure that the plastic tray pack remains sealed.
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¹ Brij is a registered trademark of ICI Americas, Inc.

² Germall is a registered trademark of Sutton Laboratories Inc.

³ Triton is a registered trademark of Rohm and Haas Co.

MATERIALS REQUIRED BUT NOT SUPPLIED WITH THE KIT

In addition to DELFIA system, the following are required:

- Tyrosine kinase and buffer for phosphorylation reaction
- Brij 35
- Precision pipettes for dispensing microlitre volumes

The DELFIA system requires the following items, which are available from PerkinElmer Life Sciences or its distributors:

1. Time-resolved fluorometer (VICTOR™ multilabel counter) plus printer and computer
2. Automatic washer - DELFIA Platewash (prod. no. 1296-026)
3. Automatic shaker - DELFIA Plateshake (prod. no. 1296-001/002 or 1296-003/004)
4. Pipette for dispensing the Enhancement Solution - Eppendorf Multipipette (prod. no. 1296-014) with 5 mL Combitips (prod. no. 1296-016) or alternatively the DELFIA Plate Dispense (prod. no. 1296-041)

WARNINGS AND PRECAUTIONS

This DELFIA Tyrosine Kinase kit is intended for research use only.

Reagents contain sodium azide (NaN_3) as a preservative. Sodium azide may react with lead and copper plumbing to form highly explosive metal azides. On disposal, flush with a large volume of water to prevent azide build-up.

Disposal of all waste should be in accordance with local regulations.

DETECTION OF TYROSINE PHOSPHORYLATION USING Eu-N1 LABELLED ANTI-PHOSPHOTYROSINE ANTIBODY

ASSAY PROCEDURE

1. Dissolve the precipitated Biotinylated poly(Glu,Ala,Tyr) Substrate in water containing 0.02 % Brij 35, e.g. in 1 mL giving 10 $\mu\text{mol/L}$ solution.
2. Perform the enzyme assay according to the instructions supplied with the enzyme. As a general rule, approx. 0.1 – 0.5 $\mu\text{mol/L}$ substrate concentration should be used in the enzyme assay, but the actual optimal level needs to be separately optimized. Note that the amount of substrate is enough for 200 assays, when using 0.5 $\mu\text{mol/L}$ substrate concentration in the enzyme assay.
3. Stop the enzyme reaction by adding appropriate amount of EDTA (if necessary).

4. Dilute the enzyme reaction solution to e.g. 1:10 or 1:50 in Assay Buffer. The optimal substrate concentration after dilution needs to be separately optimized.

The Eu counts should be between 1 000 000 and 5000 cps and blank counts between 300 and 1500 cps when measured with VICTOR multilabel counter.

5. Pipette 200 μ L of diluted reaction solution to Streptavidin plate.
6. Incubate on the DELFIA Plateshake (slow shaking) at room temperature for 1 hour (without shaking 2 hours).
7. Wash each well 2 times using DELFIA Platewash.
8. Dilute Eu-labelled anti-phosphotyrosine antibody to 1:800 into final concentration of 250 ng/mL in Assay Buffer e.g. by adding 3.75 μ L of Eu-labelled antiphosphotyrosine antibody (200 μ g/L) to 3 mL of Assay Buffer. (Enough for one strip.)
9. Pipette 200 μ L of diluted Eu-labelled antibody solution to each well.
10. Incubate on the DELFIA Plateshake (slow shaking) at room temperature for 1 hour (without shaking 2 hours).
11. Wash each well 6 times using DELFIA Platewash.
12. Add 200 μ L of Enhancement Solution directly from the reagent bottle to each well using the DELFIA Plate Dispense or Eppendorf Multipipette. When using Eppendorf Multipipette flush the Combitip first once with Enhancement Solution (to waste). Refill the Combitip and discard the first aliquot. Avoid touching the edge of the well or its contents.
13. Shake slowly for 5 minutes. The fluorescence is stable for several hours if evaporation is prevented. However, it is recommend to do the measurement within 1 hour as external factors may cause a decrease in signal with time, although this is extremely rare.
14. Measure the fluorescence in VICTOR multilabel counter.

DETECTION OF TYROSINE PHOSPHOPEPTIDE (POSITIVE CONTROL) USING Eu-N1 LABELLED ANTI-PHOSPHOTYROSINE ANTIBODY

ASSAY PROCEDURE

1. Dilute Positive Control to 1:1000 into final concentration of 2.5 nmol/L by mixing 5 μ L of Positive Control and 4995 μ L of Assay Buffer.
2. Pipette 200 μ L of Assay Buffer into wells A1-A3.
3. Pipette 8 μ L of diluted Positive Control (2.5 nmol/L) and add 192 μ L of Assay Buffer into wells A4-A6.

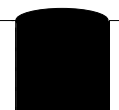
4. Pipette 20 μL of diluted Positive Control (2.5 nmol/L) and add 180 μL of Assay Buffer into wells A7-A9.
5. Pipette 40 μL of diluted Positive Control (2.5 nmol/L) and add 160 μL of Assay Buffer into wells A10-A12.
6. Incubate on the DELFIA Plateshake (slow shaking) at room temperature for 30 minutes (without shaking 1 hour).
7. Wash each well 2 times using DELFIA Platewash.
8. Dilute Eu-labelled anti-phosphotyrosine antibody to 1:800 into final concentration of 250 ng/mL in Assay Buffer e.g. by adding 3.75 μL of Eu-labelled antiphosphotyrosine antibody (200 $\mu\text{g/L}$) to 3 mL of Assay Buffer. (Enough for one strip.)
9. Pipette 200 μL of diluted Eu-labelled antibody solution to each well.
10. Incubate on the DELFIA Plateshake (slow shaking) at room temperature for 1 hour (without shaking 2 hours).
11. Wash each well 6 times using DELFIA Platewash.
12. Add 200 μL of Enhancement Solution directly from the reagent bottle to each well using the DELFIA Plate Dispense or Eppendorf Multipipette. When using Eppendorf Multipipette flush the Combitip first once with Enhancement Solution (to waste). Refill the Combitip and discard the first aliquot. Avoid touching the edge of the well or its contents.
13. Shake slowly for 5 minutes. The fluorescence is stable for several hours if evaporation is prevented. However, it is recommend to do the measurement within 1 hour as external factors may cause a decrease in signal with time, although this is extremely rare.
14. Measure the fluorescence in VICTOR multilabel counter.

Typical readings using VICTOR multilabel counter (incubations done on DELFIA Plateshake.)

Wells	Positive Control (nmol/L)	Eu-signal
A1-A3	0	515
	0	500
	0	541
A4-A6	0.10	13641
	0.10	13483
	0.10	13003
A7-A9	0.25	45282
	0.25	43239
	0.25	44481
A10-A12	0.50	235704
	0.50	210026
	0.50	226614

PROCEDURAL NOTES

1. A thorough understanding of this package insert is necessary for successful use of the DELFIA kit. The reagents supplied with this kit are intended for use as an integral unit.
2. When washing the strips, ensure that each well is filled up completely to the top edge as shown in the figure. After washing the strips, check that the wells are dry.



For detailed information on the cleaning and maintenance of the washing device, please refer to the DELFIA Platewash manual.

3. The avoidance of europium contamination and resulting high fluorescent background demands high standard pipetting and washing techniques. Avoid contaminating pipettes with Eu-labelled reagents.

The Enhancement Solution should be dispensed using only DELFIA Plate Dispense or Eppendorf Multipette after the Combitip has been first flushed with Enhancement Solution. The same Combitip should not be used for pipetting any other reagent.

When using the DELFIA Plate Dispense please refer to the manual.

LIMITATIONS OF THE PROCEDURE

Please note that it cannot be guaranteed that the biotinylated poly(Glu,Ala,Tyr) (supplied in this kit) is accepted as a substrate by all Tyrosine kinase enzymes.

WARRANTY

Purchase of the product gives the purchaser the right to use this material in his own research, development, and investigational work. The product is not to be injected into humans or used for diagnostic procedures. Wallac Oy reserves the right to discontinue or refuse orders to any customer who plans to use these products for any other purposes.

Wallac Oy does not warrant or guarantee that the product is merchantable or satisfactory for any particular purpose, nor free from any claim of foreign or domestic patent infringement by a third party, and there are no warranties, expressed or implied, to such effect. Wallac Oy will not be liable for any incidental, consequential or contingent damages involving their use including damages to the property or personal injuries.

All information supplied with the product and technical assistance given is believed to be accurate, but it remains the responsibility of the investigator to confirm all technical aspects of the application. We appreciate receiving any additions, corrections, or updates to information supplied to the customer.

PATENTS

This test system is covered by the following patents:

Europe (Austria, Belgium, Italy, Switzerland, Holland, UK, France): 0064484, 0139675

Federal Republic of Germany: P32722605-08, P3462252.7

Sweden: 8102753-4

USA: 4,565,790, 4,808,541

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