Fluorometric Assay Kit for Measuring DUSP1/MKP-1 Phosphatase Activity

CycLex Protein Phosphatase DUSP1/MKP-1 Fluorometric Assay Kit

40 Assays

Cat# CY-1373

Intended Use...1
Storage...1
Introduction 2
Principle of the Assay.............................2
Materials Provided 3
Materials Required but not Provided.......3
Precautions and Recommendations.........3-4
Detailed Protocol.....................................4-6
Evaluation of Results 7-9
Troubleshooting 10
Reagent Stability 10
References...11
Related Products......................................11-12

Intended Use

The CycLex Research product **Protein Phosphatase DUSP1/MKP-1 Fluorometric Assay Kit** is a fluorometric and non-radioactive assay designed to measure the activity of DUSP1/MKP-1 protein phosphatase. This 96-well assay is useful for screening inhibitors and modulators of DUSP1/MKP-1 activity in HTS. The kit includes all necessary components, including recombinant, human full length DUSP1/MKP-1, for use in pre-investigational drug discovery assays.

This assay kit is for research use only and not for use in human, diagnostic, or therapeutic procedures.

Storage

- Upon receipt, store the kit at -70°C.
- Don't expose reagents to excessive light.
- **AVOID REPEATED FREEZE THAW CYCLES OF "③Recombinant DUSP1/MKP-1"!**

For Reference Purpose Only!

Introduction

DUSP1 is a member of a family of dual-specificity phosphatases that dephosphorylate both phosphothreonine and phosphotyrosine residues and contain a highly conserved C-terminal catalytic domain and an N-terminal Cdc25-like (CH2) domain. DUSP1 is also known as MKP-1 (mitogenactivated protein (MAP) kinase phosphatase-1) and inactivates MAP kinase by the concomitant dephosphorylation of both its phosphothreonine and phosphotyrosine residues. The expression of DUSP1/MKP-1 is induced in human skin fibroblasts by oxidative/heat stress and growth factors.

DUSP1/MKP-1 may play an important role in the human cellular response to environmental stress as well as in the negative regulation of cellular proliferation via the dephosphorylation of MAP kinase.

A recent study* also shows that DUSP1/MKP-1 is a key factor of major depressive disorder (MDD) and it may be a new drug targets for treating depression and possibly other mood disorders.

* Duric V, Banasr M, Licznerski P, Schmidt HD, Stockmeier CA, Simen AA, Newton SS, Duman RS. A negative regulator of MAP kinase causes depressive behavior. Nat Med. 16: 1328-32, 2010.

Principle of the Assay

The **Protein Phosphatase DUSP1/MKP-1 Fluorometric Assay Kit** is based on an exclusive fluorescence substrate, OMFP (3-o-methylfluorescein phosphate). This homogenous assay kit is sensitive and convenient. This method of measurement should raise the efficiency of inhibitor screening and biochemical analysis of this enzyme.

Summary of Procedure

Mix 40 µL of Assay mixture and 5 µL of test compound in the wells

Add 5 µL of Recombinant DUSP1/MKP-1

Incubate for 20 min at room temp.

Add 25 µL of Stop Solution

Measure fluorescence at 510-540 nm emission / 482-502 nm excitation

For Reference Purpose Only!

Materials Provided

All samples and standards should be assayed in duplicate. The following components are supplied and are sufficient for forty assays.

Components of Kit

Components	Quantity	Storage
①10X DUSP1/MKP-1 Assay Buffer	600 µL x 1	Below -20°C
②10X OMFP	550 µL x 1	Below -20°C
③Recombinant DUSP1/MKP-1 (20 units/µL)*	250 µL x 1	-70°C
④10X Phosphatase Inhibitor : 100 mM Na_3VO_4 in DW	500 µL x 1	Below -20°C
⑤Stop Solution	1,300 µL x 2	Below -20°C
⑥Instruction Manual	1	Room temp.

* The GenBank Accession number of DUSP1/MKP-1 gene is NM_004417. "③Recombinant DUSP1/MKP-1", expressed in *E. coil*, contains a region of dual specificity phosphatases (amino acids: 173-309) and an N-terminal GST tag.

Materials Required but not Provided

- **Microtiter plate suitable for use with a fluorometric plate reader**
- **Fluorometric plate reader or microtiter plate fluorometer:** Use a fluorescence microplate reader equipped with appropriate filters. OMFP has excitation/emission maxima of approximately 485/525 nm. We have found that standard filters for blue-fluorescent dyes (e.g., excitation = 485 ±12.5 nm, emission = 525 ± 20 nm) can be used to detect OMFP.
- **Pipettors:** 2-20 µL, 20-200 µL and 200-1000 µL precision pipettors with disposable tips
- **Multi-channel pipette**
- **Microtiter plate shaker**
- **Distilled water** (DW) or equivalent high quality water
- **Microcentrifuge and tubes** for sample preparation
- **Reagent reservoirs**
- **Ice bucket** to keep reagents cold until use

Precautions and Recommendations

- Upon receipt, store the kit at -70°C.
- Do not expose reagents to excessive light.
- Do not use kit components beyond the indicated kit expiration date.
- Rinse all detergent residue from glassware.
- Use deionized water of the highest quality.
- Do not mix reagents from different kits.
- Do not mouth pipette or ingest any of the reagents.

For Reference Purpose Only!

- Do not smoke, eat, or drink when performing the assay or in areas where samples or reagents are handled.

NOTE: THE FOLLOWING PROCEDURES ARE INTENDED ONLY AS A GUIDELINE. THE OPTIMAL EXPERIMENTAL CONDITIONS WILL VARY DEPENDING ON THE PARAMETERS BEING INVESTIGATED, AND MUST BE DETERMINED BY THE INDIVIDUAL USER.

Detailed Protocol

Preparation of Reagents

Thaw the reagents at room temperature except **"③Recombinant DUSP1/MKP-1**" and keep all reagents including "**③Recombinant DUSP1/MKP-1**" on ice until use. **AVOID REPEATED FREEZE THAW CYCLES OF "③Recombinant DUSP1/MKP-1"!** Making aliquot of "**③Recombinant DUSP1/MKP-1**" is recommended. Use them only after they are completely thawed and mixed.

Prepare **Assay Mixture** by adding 5 µL of the **①10X DUSP1/MKP-1 Assay Buffer** and 5 µL of the **10X OMFP** to 30 µL of distilled (deionized) water per one assay. Mix well.

Assay Mixture

Assay reagents	1 assay	8 assays	16 assays	32 assays	48 assays
Distilled water **①10X DUSP1/MKP-1 Assay Buffer** **②10X OMFP**	**30 µL** **5 µL** **5 µL**	**240 µL** **40 µL** **40 µL**	**480 µL** **80 µL** **80 µL**	**960 µL** **160 µL** **160 µL**	**1,440 µL** **240 µL** **240 µL**
Total volume of Assay Mixture	**40 µL**	**320 µL**	**640 µL**	**1,240 µL**	**1,920 µL**

For Reference Purpose Only!

Assay Procedure

In order to estimate the inhibitory effect on DUSP1/MKP-1 activity by the test compounds correctly, it is necessary to conduct the control experiment of "**Vehicle control**" at least once for every experiment and "**Inhibitor control**" at least once for the first experiment, in addition to "**Test sample**" as indicated in the Table.1 (below). When test chemicals cause an inhibitory effect on DUSP1/MKP-1 activity, the level of increase of fluorescence intensity is weakened as compared with "**Vehicle control**". The increase in fluorescence intensity is not observed in "**Inhibitor control**".

1. Following Table.1 below, first, add **"Assay mixture"** to microtiter plate wells. Second, add **"Test Compound"** or **"Vehicle of Test Compounds"** or **"10X Phosphatase Inhibitor"** to each well of the microtiter plate and mix well.

Table.1: Reaction mixture

Assay reagents	Test Sample	Vehicle Control	Inhibitor Control	No Enzyme Control
Assay Mixture	**40 µL**	**40 µL**	**40 µL**	**40 µL**
Test Compound **Vehicle of Test Compounds** **④10X Phosphatase Inhibitor**	**5 µL** **-** **-**	**-** **5 µL** **-**	**-** **-** **5 µL**	**-** **5 µL** **-**
③Recombinant DUSP1/MKP-1 **Distilled water**	**5 µL** **-**	**5 µL** **-**	**5 µL** **-**	**-** **5 µL**
Total Volume of the Reaction mixture	**50 µL**	**50 µL**	**50 µL**	**50 µL**

2. Initiate reactions by adding **5 µL of "③Recombinant DUSP1/MKP-1"** or distilled water to each well and mixing thoroughly at room temperature.

3. Incubate for 20 min or desired length of time at room temperature.

4. Add **25 µL of "⑤Stop Solution"** to each well of the microtiter plate, and mix thoroughly.

5. Measure fluorescence intensity using a microtiter plate fluorometer with excitation at 482-502 nm and emission at 510-540 nm.

6. The efficacy of the Test compound is the difference in fluorescence intensity between **"Vehicle control"** and **"Test sample"**.

Note: If necessary, it is possible to store the microtiter plate after adding **"⑤Stop Solution"** for a few hours at 4°C. The microtiter plate must be sealed to prevent evaporation and kept from excessive light.

For Reference Purpose Only!

Alternate procedure

1'. Following Table.1 above, first, add **"Assay mixture"** to microtiter plate wells. Second, add **"Test Compound"** or **"Vehicle of Test Compounds"** or **"④10X Phosphatase Inhibitor"** to each well of the microtiter plate and mix well.

2'. Initiate reactions by adding 5 µL of **"③Recombinant DUSP1/MKP-1"** or distilled water to each well and mixing thoroughly at room temperature.

3'. Read fluorescence intensity for 20 to 60 min at 1 to 2 min intervals using microtiter plate fluorometer with excitation at 482-502 nm and emission at 510-540 nm.

4'. Measure and calculate the rate of reaction while the reaction velocity remains constant.

Caution and Significance

• All samples and "Recombinant DUSP1/MKP-1" should be assayed in duplicate.

• Use of a microtiter plate shaker is recommended for complete mixing.

• If the test compounds or samples themselves emit fluorescence at excitation wavelength: 482-502 nm and fluorescence wavelength: 510-540 nm, the test assay cannot be evaluated correctly.

For Reference Purpose Only!

Evaluation of Results

Analysis of Inhibitor Effect

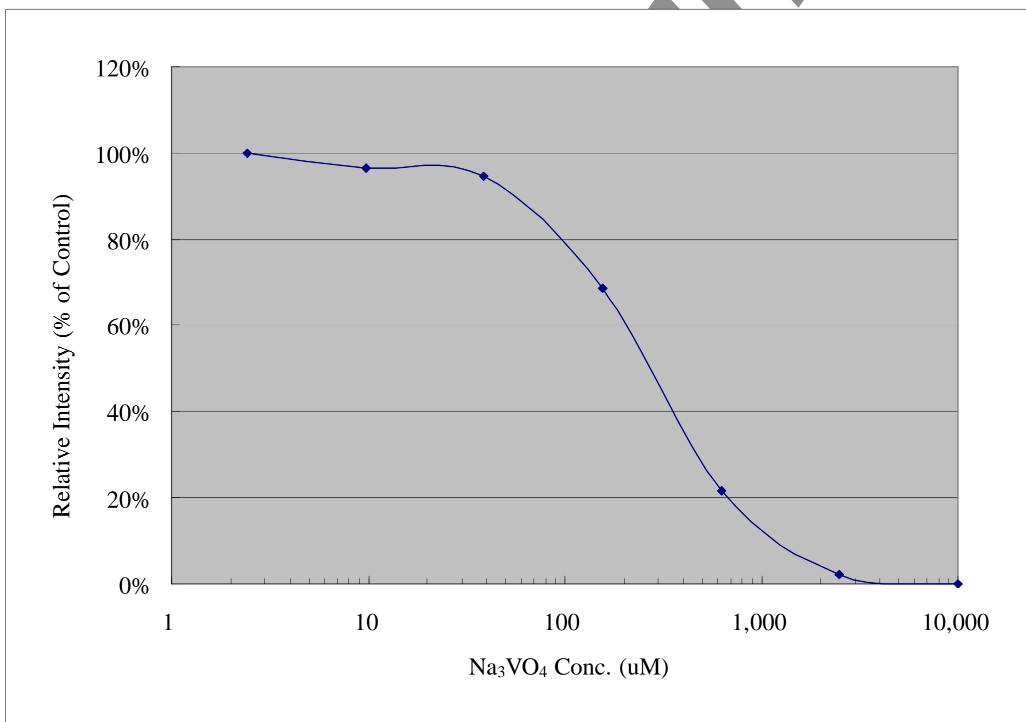
% Intensity

1. Run reactions with test compounds and Vehicle as described in the **Detailed Protocol**.
2. Subtract fluorescence intensity of "No Enzyme Control" from all experimental samples (Test Samples and Vehicle Control).
3. Calculate the % Intensity:

$$\% \text{ Intensity} = \frac{\text{Fluorescence Intensity of Test Sample}}{\text{Fluorescence Intensity of Vehicle Control}} \times 100$$

Note: This % Intensity is a rough value of enzyme activity or inhibition. For greater accuracy, plot a standard curve of DUSP1/MKP-1 for each new set of reactions and estimate the % Activity (see below).

Fig.1 DUSP1/MKP-1 Inhibition Curve by Na_3VO_4 (SOV; Sodium Orthovanadate)



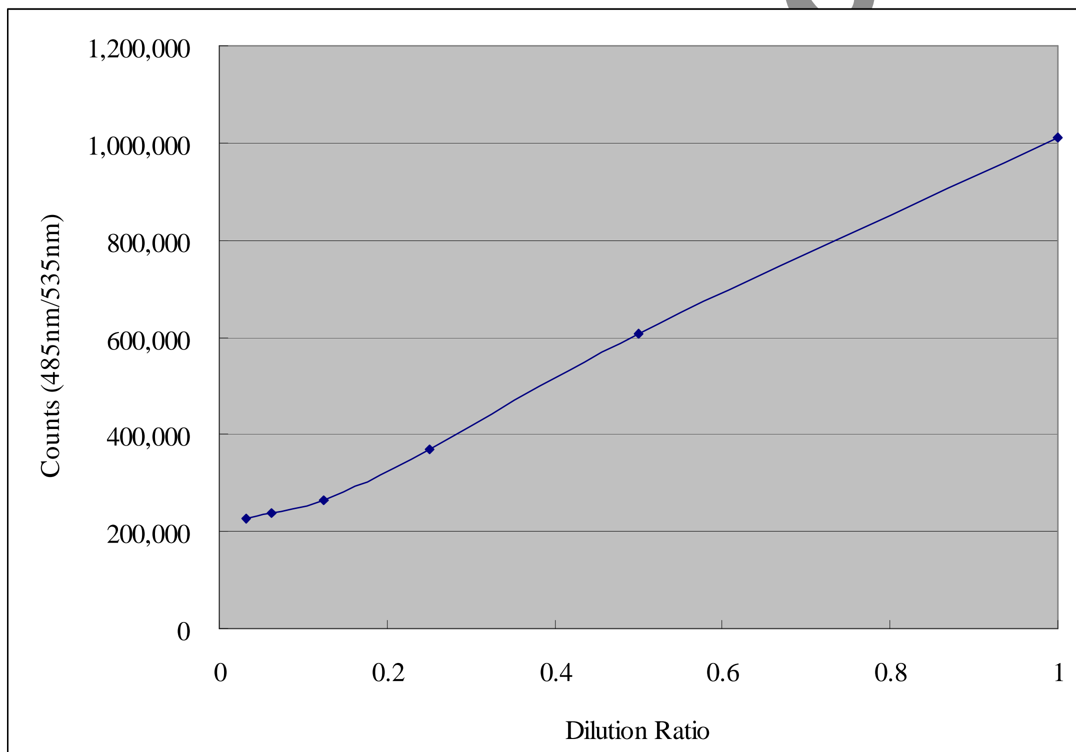
For Research Purpose Only!

Analysis of Enzyme Activity

DUSP1/MKP-1 Standard Curve and % Activity

1. Dilute the "**①10X DUSP1/MKP-1 Assay Buffer"** 1:10 with distilled water to make 1X Assay Buffer.
2. Make serial dilutions of **"③Recombinant DUSP1/MKP-1"** with 1X Assay Buffer (ex. 100%, 25%, 6.25%, 1.56% and 0%).
3. Run reactions with Vehicle and serial dilutions of Recombinant DUSP1/MKP-1 as described in the **Detailed Protocol**.
4. Plot standard curve data (dose dependent curve data) as fluorescence intensity at 510-540 nm versus dose of DUSP1/MKP-1 (ng/assay).
5. Obtain a line-fit to the data using appropriate calculations.
6. Use the slope and Y-intercept to calculate the amount of DUSP1/MKP-1 activity for the experimental data.

Fig.2 Dose Dependency of Recombinant DUSP1/MKP-1 (at 20 minutes reaction)



For Reference Purpose Only!

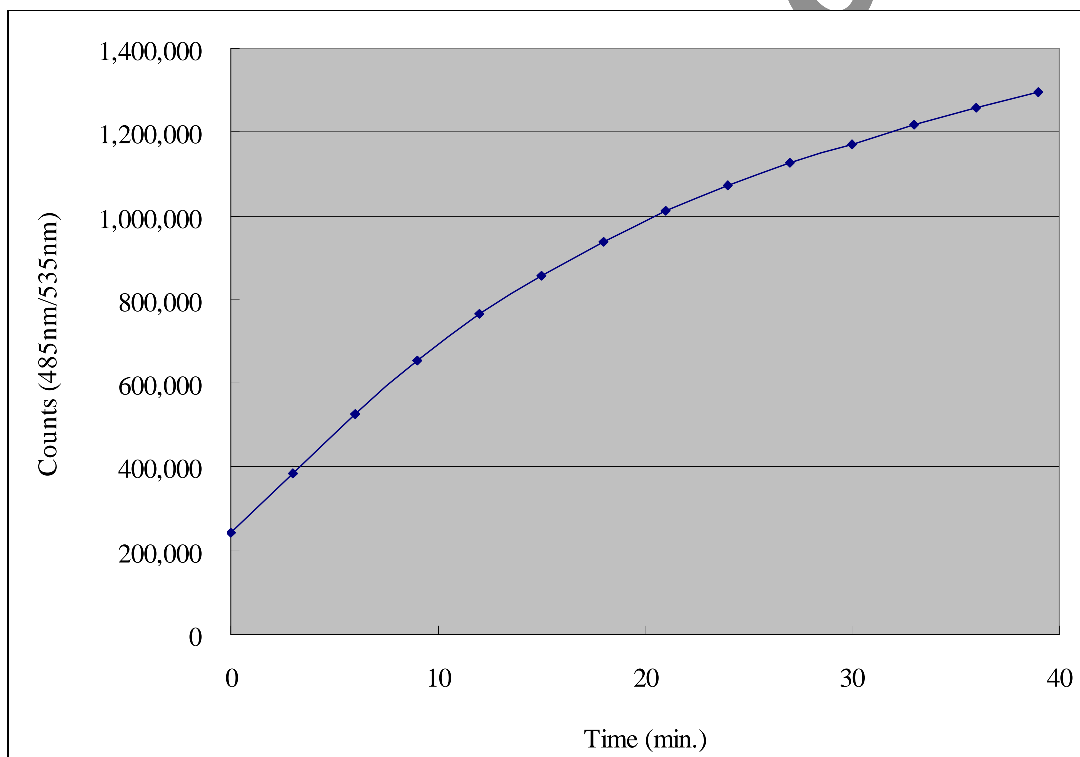
Analysis of Kinetics

Time Course Curve

1. Run reactions as described in the *Detailed Protocol*.
2. Subtract fluorescence intensity at the 0 time from all reaction time points.
3. Plot fluorescence intensity at 510-540 nm versus reaction time.
4. Determine the reaction time range in which the increase in fluorescence intensity at 510-540 nm is linear.
5. Calculate activity:

$$\text{Activity (reaction velocity)} = \frac{\text{Fluorescence Intensity of Test Sample}}{\text{Reaction time (min.)}}$$

Fig.3 Time Course Curve of Recombinant DUSP1/MKP-1



For Reference Purpose Only!

Troubleshooting

1. The Recombinant DUSP1/MKP-1 should be run in duplicate using the protocol described in the *Detailed Protocol*. Incubation times or temperatures significantly different from those specified may give erroneous results.

2. The reaction curve is nearly a straight line if the kinetics of the assay is of the first order. Variations in the protocol can lead to non-linearity of the curve, as can assay kinetics of other than first order. For a non-linear curve, point to point or quadratic curve fit methods should be used.

3. Poor duplicates, accompanied by elevated values for wells containing no sample, indicate inaccurate dispensing of assay reagents. If all instructions in the *Detailed Protocol* were followed accurately, such results indicate a need for multi-channel pipette maintenance.

Reagent Stability

All of the reagents included in the **Protein Phosphatase DUSP1/MKP-1 Fluorometric Assay Kit** have been tested for stability. Reagents should not be used beyond the stated expiration date. Upon receipt, should be stored at -70°C. Avoid repeated freeze-thaw cycles of "③ Recombinant DUSP1/MKP-1". After use, return kit reagents to -70°C as soon as possible.

For research use only, not for use in human, diagnostic or therapeutic procedures

For Reference Purpose Only!

References

1. Alessi, D. R., Smythe, C., Keyse, S. M. The human CL100 gene encodes a tyr/thr-protein phosphatase which potently and specifically inactivates MAP kinase and suppresses its activation by oncogenic ras in Xenopus oocyte extracts. Oncogene 8: 2015-2020, 1993.

2. Bhalla, U. S., Ram, P. T., Iyengar, R. MAP kinase phosphatase as a locus of flexibility in a mitogen-activated protein kinase signaling network. Science 297: 1018-1023, 2002.

3. Brondello, J.-M., Pouyssegur, J., McKenzie, F. R. Reduced MAP kinase phosphatase-1 degradation after p42/p44(MAPK)-dependent phosphorylation. Science 286: 2514-2517, 1999.

4. Keyse, S. M., Emslie, E. A. Oxidative stress and heat shock induce a human gene encoding a protein-tyrosine phosphatase. Nature 359: 644-647, 1992.

4. Patterson, K. I., Brummer, T., O'Brien, P. M., Daly, R. J. Dual-specificity phosphatases: critical regulators with diverse cellular targets. Biochem. J. 418: 475-489, 2009.

5. Wang, X., Meng, X., Kuhlman, J. R., Nelin, L. D., Nicol, K. K., English, B. K., Liu, Y. Knockout of Mkp-1 enhances the host inflammatory responses to Gram-positive bacteria. J. Immun. 178: 5312-5320, 2007.

6. Calvisi DF, Pinna F, Meloni F, Ladu S, Pellegrino R, Sini M, Daino L, Simile MM, De Miglio MR, Virdis P, Frau M, Tomasi ML, Seddaiu MA, Muroni MR, Feo F, Pascale RM. Dual-specificity phosphatase 1 ubiquitination in extracellular signal-regulated kinase-mediated control of growth in human hepatocellular carcinoma. Cancer Res. 68: 4192-200, 2008.

7. Duric V, Banasr M, Licznerski P, Schmidt HD, Stockmeier CA, Simen AA, Newton SS, Duman RS. A negative regulator of MAP kinase causes depressive behavior. Nat Med. 16: 1328-32, 2010.

Related Products

*** CycLex Protein Tyrosine Phosphatase 1B (PTP1B) Fluorometric Assay Kit: Cat# CY-1350**
*** CycLex T Cell Protein Tyrosine Phosphatase (TC-PTP) Fluorometric Assay Kit: Cat# CY-1351**
*** CycLex Protein Phosphatase Cdc25A Fluorometric Assay Kit: Cat# CY-1352**
*** CycLex Protein Phosphatase Cdc25B Fluorometric Assay Kit: Cat# CY-1353**
*** CycLex Protein Phosphatase Cdc25C Fluorometric Assay Kit: Cat# CY-1354**
*** CycLex Protein Phosphatase Cdc25 Combo Fluorometric Assay Kit: Cat# CY-1355**
*** CycLex Protein Phosphatase Cdi1/KAP Fluorometric Assay Kit: Cat# CY-1356**
*** CycLex Protein Phosphatase LMW-PTP/ACP1 Fluorometric Assay Kit: Cat# CY-1358**
*** CycLex Protein Phosphatase DUSP1/MKP-1 Fluorometric Assay Kit: Cat# CY-1373**

*** Protein Tyrosine Phosphatase PTPRA 1st Catalytic Domain: Cat# CY-E1301**
*** Protein Tyrosine Phosphatase PTPRA 2nd Catalytic Domain: Cat# CY-E1302**
*** Protein Tyrosine Phosphatase PTPRD 2nd Catalytic Domain: Cat# CY-E1307**
*** Protein Tyrosine Phosphatase PTPRE 1st Catalytic Domain: Cat# CY-E1308**
*** Protein Tyrosine Phosphatase PTPRF 1st Catalytic Domain: Cat# CY-E1310**
*** Protein Tyrosine Phosphatase PTPRK 1st Catalytic Domain: Cat# CY-E1316**
*** Protein Tyrosine Phosphatase PTPRQ: Cat# CY-E1323**
*** Protein Tyrosine Phosphatase PTP4A2: Cat# CY-E1341**

For Reference Purpose Only!

*** Recombinant Cdc25A (Catalytic domain): Cat# CY-E1352**
*** Recombinant Cdc25B (Catalytic domain): Cat# CY-E1353**
*** Recombinant Cdc25C (Catalytic domain): Cat# CY-E1354**
*** Recombinant Cdi1/KAP: Cat# CY-E1356**
*** Protein Phosphatase PP5: Cat# CY-E1359**
*** Protein Tyrosine Phosphatase PTPN3/PTPH1: Cat# CY-E1360**
*** Protein Tyrosine Phosphatase PTPN6/SHP-1: Cat# CY-E1363**
*** Protein Tyrosine Phosphatase PTPN7/HePTP: Cat# CY-E1364**
*** Protein Tyrosine Phosphatase PTPN8/PTPN22: Cat# CY-E1365**
*** Protein Tyrosine Phosphatase PTPN9/MEG2: Cat# CY-E1366**
*** Protein Tyrosine Phosphatase PTPN11/SHP-2: Cat# CY-E1367**
*** Protein Tyrosine Phosphatase PTPN12/PTP-PEST: Cat# CY-E1368**
*** Protein Tyrosine Phosphatase PTPN13/FAP-1: Cat# CY-E1369**
*** Protein Tyrosine Phosphatase PTPN14/PEZ: Cat# CY-E1370**
*** Protein Tyrosine Phosphatase PTPN21/PTPD1: Cat# CY-E1372**
*** Protein Phosphatase DUSP1/MKP-1: Cat# CY-E1373**

PRODUCED BY

CycLex Co., Ltd.
1063-103 Terasawaoka
Ina, Nagano 396-0002
Japan
Fax: +81-265-76-7618
e-mail: info@cyclex.co.jp
URL: http://www.cyclex.co.jp

CycLex/CircuLex products are supplied for research use only. CycLex/CircuLex products and components thereof may not be resold, modified for resale, or used to manufacture commercial products without prior written approval from CycLex Co., Ltd.. To inquire about licensing for such commercial use, please contact us via email.

For Reference Purpose Only!