Enzymatic Assay of FK-BINDING PROTEIN
Peptidyl-Prolyl Isomerase Activity

PRINCIPLE:

Suc-Ala-Leu-cis-Pro-Phe-pNA —FKBP→ Suc-Ala-Leu-trans-Pro-Phe-pNA

Suc-Ala-Leu-trans-Pro-Phe-pNA + H_2O —Chymotrypsin→ Suc-Ala-Leu-trans-Pro-Phe + p-Nitroaniline

Abbreviations used:
pNA = p-Nitroanilide
FKBP = FK Binding Protein

CONDITIONS: T = 1°C, pH 8.0, A_{405nm}, Light path = 1 cm

METHOD: Continuous Spectrophotometric Rate Determination

REAGENTS:

A. 50 mM HEPES Buffer with 100 mM Sodium Chloride, pH 8.0 at 1°C
(Prepare 100 ml in deionized water using HEPES, Free Acid, Sigma Prod. No. H-3375 and Sodium Chloride, Sigma Prod. No. S-9625. Adjust to pH 8.0 at 1°C with 1 M NaOH.)

B. 2,2,2-Trifluoroethanol (TFE)
(Use 2,2,2-Trifluoroethanol, Sigma Prod. No. T-8132. Before use, dry overnight with Calcium Chloride, Anhydrous, Sigma Prod. No. C-4901 and Molecular Sieves, 3Å, Sigma Prod. No. M-2010.[1] Filter through a Whatman #5 filter paper in a dry room.)

C. 470 mM Lithium Chloride Solution (LiCl/TFE)
(Prepare 10 ml in Reagent B using Lithium Chloride, Anhydrous, Sigma Prod. No. L-0505. Dry the Lithium Chloride before use in a vacuum oven at 150°C for 24 hours. The Lithium Chloride Solution is at or near saturation at 460 mM in Reagent B. If the solution is turbid or not all of the Lithium Chloride goes into solution with stirring (1 hour) then filter through a Whatman #1 filter paper before use. **PREPARE FRESH.**)

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REAGENTS:

D. 6.0 mM Suc-Ala-Leu-Pro-Phe-pNA Solution (Substrate)
(Immediately before use, prepare 0.5 ml in Reagent C using Suc-Ala-Leu-Pro-Phe-pNA, Bachem Biosciences Inc. Prod. No. L-1620. Keep on ice.)[2]

E. 1 mM Hydrochloric Acid Solution (HCl)
(Prepare 10 ml in deionized water using Hydrochloric Acid, Sigma Prod. No. H-7020.)

F. Chymotrypsin Enzyme Solution (Chym)
(Immediately before use, prepare a solution containing 60 mg/ml of a-Chymotrypsin, Sigma Prod. No. C-4129 in cold Reagent E.)

G. FK Binding Protein Solution (FKBP)
(Immediately before use, prepare a solution containing 1 unit/ml of FK Binding Protein in cold Reagent A.)

PROCEDURE:

Pipette (in milliliters) the following reagents into suitable cuvettes:

	Test	Blank
Reagent A (Buffer)	0.865	0.875
Reagent G (FKBP)	0.01	------
Reagent F (Chym)	0.10	0.10

Mix by inversion and equilibrate to 1°C. Monitor the A_{405nm} until constant, using a suitably thermostatted spectrophotometer. Then add:

	Test	Blank
Reagent D (Substrate)	0.025	0.025

Immediately mix by inversion and record the increase in A_{405} for approximately 1 minute.[3] Obtain the r A_{405nm}/minute using the maximum linear rate for both the Test and Blank.

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CALCULATIONS:

$$\text{Units/ml enzyme} = \frac{(\text{r } A_{405nm}/\text{min Test} - \text{r } A_{405nm}/\text{min Blank})(1)(df)}{(0.01)(9.3)}$$

1 = Total volume (in milliliter) of assay
df = Dilution factor
0.01 = Volume (in milliliter) of FKBP used
9.3 = Millimolar extinction coefficient[4] of p-Nitroaniline at 405 nm under the conditions of the assay

$$\text{Units/mg solid} = \frac{\text{units/ml enzyme}}{\text{mg solid/ml enzyme}}$$

$$\text{Units/mg protein} = \frac{\text{units/ml enzyme}}{\text{mg protein/ml enzyme}}$$

UNIT DEFINITION:

One unit will catalyze the isomerization of 1.0 micromole of Suc-Ala-Leu-cis-Pro-Phe-p-Nitroanilide to Suc-Ala-Leu-trans-Pro-Phe-p-Nitroanilide per minute at pH 8.0 at 1°C.

FINAL CONCENTRATION:

In a 1.00 ml reaction mix, the final concentrations are 44 mM HEPES, 88 mM sodium chloride, 0.15 mM Suc-Ala-Leu-Pro-Phe-p-nitroanilide, 12 mM lithium chloride, 2.5% (v/v) 2,2,2-trifluoroethanol, 0.1 mM hydrochloric acid, 6 mg chymotrypsin, and 0.01 unit FK-binding protein.

REFERENCE:

Kofron, J.L., Kuzmi_, P., Kishore, V., Colón-Bonilla, E., and Rich, D.H. (1991) *Biochemistry* **30**, 6127-6134

NOTES:

1. In order to dry Reagent B (TFE), add the calcium chloride and molecular sieves each, at a concentration of 10% (w/v).

2. The presence of Reagent C (LiCl/TFE) insures that the majority of the substrate is converted to the cis

isomer.

NOTES: (continued)

3. A sharp increase in absorbance ("burst phase") will occur almost instantly after addition of the substrate as the chymotrypsin hydrolyzes the excess trans isomer. The cis to trans conversion catalyzed by the FK Binding Protein is mostly linear within the first minute of the reaction after the "burst phase."

4. This value was experimentally determined by Sigma.

5. This assay is based on the cited reference.

6. Where Sigma Product or Stock numbers are specified, equivalent reagents may be substituted.

This procedure is for informational purposes. For a current copy of Sigma's quality control procedure contact our Technical Service Department.