Cut-N-Glow™ Factor Xa Protease Activity Assay Kit

Product Number: 29004001

PRODUCT INSERT

INTENDED USE: *FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES*

Cut-N-Glow is the first fully biological *in vivo* protease mapping tool that emits fluorescence. This assay is easily tailored via standard cloning techniques to detect specific proteases or protease inhibitors or to map protease substrate preference *in vitro* or i*n vivo*. Chemical synthesis is not required and there is no need for co-factors or co-substrates. Additionally, this assay only requires two reagents and both are proteins that can be easily obtained following over-expression of *E. coli.*

BACKGROUND AND PRINCIPLE OF THE TEST

Background:

Proteases occur naturally in all organisms and are valuable tools in medical diagnostics serving as initiators of cell signaling, as regulators of immune responses, and as agents of infectious disease. Therefore, mapping proteases in parasitic diseases and bacteria as well as assayable proteases associated with cancer could lead to the identification of shared structural similarities validating potential drug targets. This strategy utilizes split proteins in a conditionally inactive form with the aid of a conformational distortion maintained by a cleavable tether. This method is applied to convert split GFP into a latent fluorophore that can be activated by site-specific proteolysis. The chimeric GFP serves as a substrate for representative enzymes from the three major protease classes: serine, cysteine, and aspartic acid.

Factor X, or prothrombinase, is a serine endopeptidase of the coagulation cascade. Factor X is activated into factor Xa, which functions to cleave prothrombin to yield thrombin. Thrombin acts as a serine protease that catalyzes the conversion of soluble fibrinogen into insoluble strands of fibrin, in addition to catalyzing many other coagulation-related reactions.

This assay kit is specific for Factor Xa activity and contains sufficient reagents for one 96-well plate (96 tests).

Assay

The Cut-N-Glow™ approach involves the introduction of a structural distortion into one of the complementary fragments (GFP 11), through the use of a conditionally stable tether, which serves to constrain the N and C termini of GFP11 closely in space, thereby diminishing the mutual affinity of the two fragments and blocking

protein self-assembly until the tether is cleaved. The distortion can be reversed upon proteolysis of the tether, resulting in fragment assembly with GFP 1-10, generating reconstituted, functional GFP.

REAGENTS

Components Supplied: *(Sufficient reagents have been supplied for 96 individual tests)*

- ***Constrained substrate***, 1.0 mL. Supplied ready to use at approximately 225 µg/mL. Store -20°C.
- ***Detector (S1-10*)**: Complementary GFP fragment. 20 mL. Supplied ready to use at approximately 1.0 mg/mL.. Store -20°C.
- ***Positive Control Reagent***, 500 µL. Supplied ready to use. Store -20°C.

Note: When stored at -20°C, the reagents are stable until the date indicated either on the box or on each component. Depending on the particular usage requirements, it may be appropriate to re-aliquot reagents to smaller working volumes to avoid repeated freeze-thawing or repeated pipetting from the same vial.

Materials required, but not supplied:

- Recommended Factor Xa, Sigma-Aldrich, cat# F9302-50UG, from bovine plasma
- 96 well microplate compatible with customer's fluorescence plate reader.
- UV plate reader
- Humidified incubation system

In Vitro Complementation assay

1. Equilibrate kit components to ambient temperature.
2. In a microplate well, mix 10 µL constrained substrate with 200 µL of detection reagent, and allow to equilibrate at ambient temperature for 2 h. Repeat for the number of wells as needed.
3. Add 0.5 – 1.0 µL of thrombin to reagent control well.
4. Add 1.0-10 µL of test sample to remaining wells.
5. Incubate overnight at 37ºC in a humidified incubator, if necessary.
6. Measure fluorescence at 535 (± 25) nm using 485 (±25) nm excitation.

DATA ANALYSIS

Data Analysis

Subtract the blank fluorescence values from the final fluorescence values of the sample(s) and the positive control. Perform appropriate statistical analysis, if applicable.

REFERENCES

1. Protease Activation of Split Green Fluorescent Protein. Callahan, B.P., Stanger, M.J., Belfort, M. *ChemBioChem* - 2010 Nov 2;11(16):2259-63

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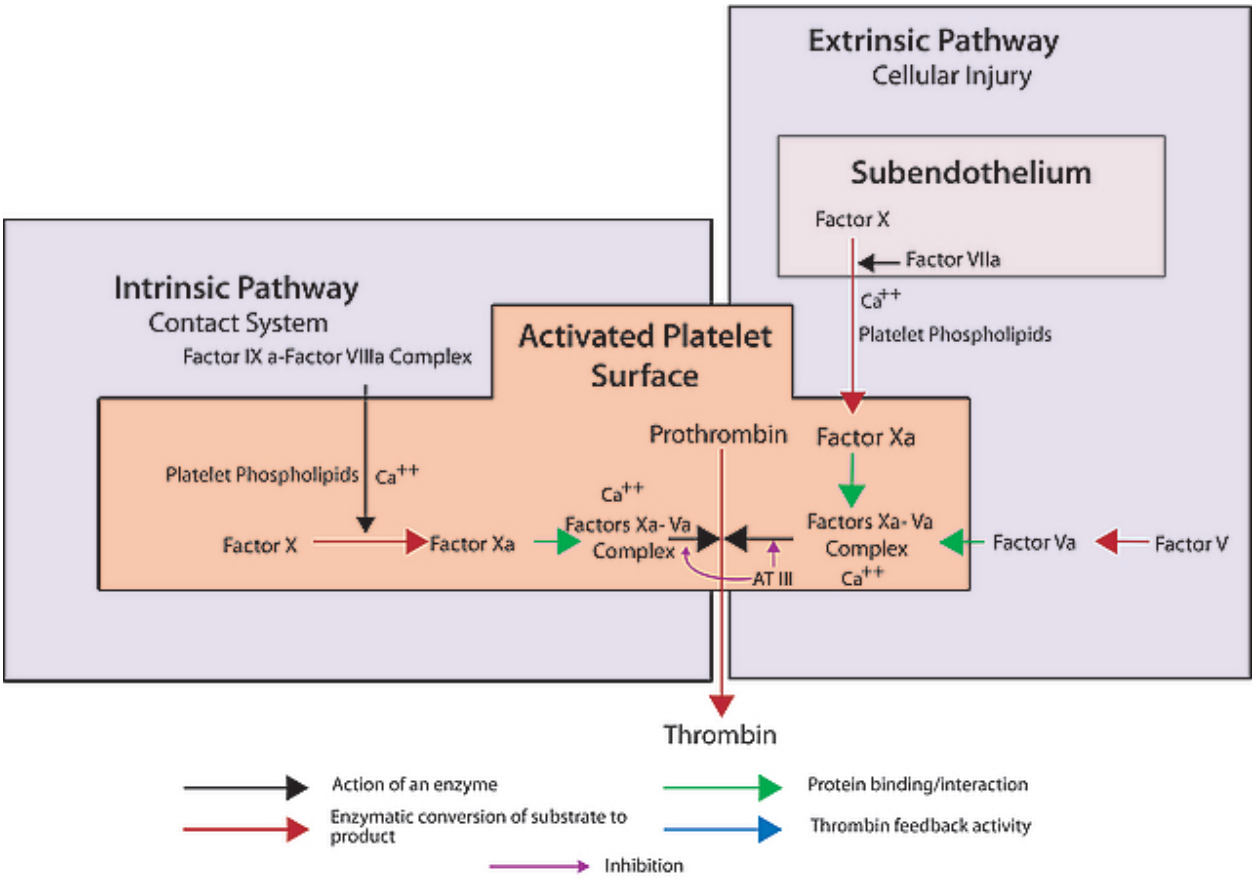
Extrinsic Pathway
Cellular Injury
Subendothelium
Factor X
Factor VIIa
Ca++
Platelet Phospholipids
Intrinsic Pathway
Contact System
Factor IX a-Factor VIIIa Complex
Activated Platelet Surface
Prothrombin
Factor Xa
Platelet Phospholipids
Ca++
Ca++
Factor X
Factor Xa
Factors Xa- Va Complex
Factors Xa- Va Complex
Factor Va
Factor V
AT III
Ca++
Thrombin
Action of an enzyme
Protein binding/interaction
Enzymatic conversion of substrate to product
Thrombin feedback activity
Inhibition