GFP “Fold ‘n’ Glow” Protein Solubility Assay Kit

Product Number 20004001

PRODUCT INSERT

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

The “Fold ‘n’ Glow” Protein Solubility kit allows a test protein to be expressed as an N-terminal fusion with GFP and will determine the amount of protein that is properly folded in a given sample as the folding reporter gives a signal directly proportional to the amount of correctly folded protein[1]. The kit can be used for the detection and quantification of any protein by tagging and detecting either soluble or insoluble proteins. The kit contains sufficient reagents for one 96-well plate (96 tests).

BACKGROUND AND PRINCIPLE OF THE TEST

Background:

Green fluorescent protein (GFP) fusions and split protein tags are widely used for the analysis of protein. These large tags can perturb protein solubility, misfold, and alter the protein’s processing. The split GFP technology used in the Fold ‘n’ Glow assay overcomes these problems. The protein tag is a genetically encoded split GFP technology, engineered with small, soluble, self-associating GFP fragments. Thus, it is a simple split GFP system that doesn’t change fusion protein solubility, or require chemical ligation, fused interacting partners, co-expression or co-refolding. Furthermore, while fluorogenic biarsenical FlaSH or ReASH substrates also overcome these limitations, they also require many other conditions not necessary when using the split GFP technology. The GFP system is a simple and easy tagging and detection system[2]. This kit may be used: to quantify the expression level of the tagged protein; to determine the solubility of a protein; or to determine the solubility of a protein’s domain.

The assay:

The kit is a protein tagging and detection method that uses split GFP technology in a fluorescent complementation assay. The protein to be quantified is fused to a small GFP fragment (contained in the S11plasmid) via a flexible linker. Expressed separately, neither the fusion protein of interest nor the GFP detector (S1-10) is fluorescent. When mixed, the properly folded fusion protein and detector spontaneously associate, formatting the fluorophore. Misfolding or aggregation of the fusion protein make the GFP tag inaccessible and prevent complementation, thus preventing fluorescence. Therefore, misfolded or aggregated proteins are not included in the quantification of the protein of interest.

REAGENTS

Components Supplied: *(Sufficient reagents have been supplied for 84 tests)*

Detector (S1-10): Complementary GFP fragment. Supplied ready to use. Store -20°C.

Positive control (SRS11): Positive control GFP fusion protein. Supplied ready to use at 5µM. Store -20°C.

Plasmid (S11) : Kanamycin resistance (Kan^R). *BamHI* and *NdeI* restriction sites. Supplied at 100ng/mL Store -20°C.

Note: The stability of the components included in this kit is approximately 6 months when stored at -20°C. When stored properly, the reagents are stable until the date indicated either on the box or 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:

- Competent expression cells (BL2/DE3)
- Kanamycin
- IPTG (Isopropyl β-D-1-thiogalactopyranoside)
- LB growth media and plates
- *NdeI* And *BamHI* restriction enzymes
- Ligation materials
- Metal affinity column
- TNG Buffer (50mM Tris pH 7.4, 0.1M NaCl, 10% glycerol)
- Bovine serum albumin
- Plasmid Isolation Reagents
- 96 well plate
- 4°C refrigerator
- -20°C freezer
- Incubator
- Centrifuge(s) and appropriate size tubes
- Sonicator
- Microplate fluorescence reader
- Vortex mixer
- Water bath
- Graduated cylinders and assorted beakers
- Pipettes and tips
- Disposable gloves

A. Preparation of insert DNA

Prior to performing the assay, carefully read all instructions.

1. Perform plasmid prep and/or PCR. Use standard materials/protocol not included.
2. Use *NdeI* (5') and *BamHI* (3') restriction sites to perform a restriction digest that generates overhangs on the DNA insert. *Optional:* Purify digest fragment from agarose gel.

B. Preparation of S11 Vector

1. To ensure that you have a renewable source of plasmid DNA, transform the plasmid vector provided in this kit in an *E.coli* host strain.
    - It is recommended that bacterial frozen stocks be prepared of all transformed plasmids using standard molecular biology techniques.
2. Purify plasmid DNA for cloning using Plasmid Preparation kits or other techniques (not included).
3. Perform restriction enzyme digest of the S11 vector using *Nde1* and *BamH1*to prepare the plasmid for the insert DNA. Follow the manufacturer's instructions for use of the enzymes. Leave sticky ends over hangs in preparation for ligation.
4. *Optional:* Dephosphorylate the digest to decrease non-recombinant background. Use molecular grade calf intestinal or shrimp alkaline phosphatase according to the manufacturer's directions.
5. Perform ligation reaction according to manufacturers' instructions.
6. Store vector at –20°C until used.

C. Clone DNA insert as an N-terminal fusion into S11 vector

1. Ligate the DNA insert with the digested S11 using standard DNA ligation protocol and manufacturer's protocol resulting in a S11 fusion plasmid.
2. Transform the S11-fusion plasmid in an expression host for high yields of quality plasmid. Use standard methods based on the screening host used.
3. Identify the positive clones using standard methods. Note: IPTG / X-gal screening is effective in the first 24 hours post plating as the T7 promoter is highly active and absorbs resources from the LacZ gene.
4. Perform plasmid DNA purification, sequence to verify reading frame, or use *in vitro* transcription/translation.

D. Preparation of S11 fusion protein

1. Prepare cells and extract *soluble* S11 fusion proteins.
   a. Grow a 200ml culture of bacteria, transformed S11 fusion protein, in LB growth medium and Kanamycin [20-50μg/ml] to log phase at OD $_{600nm}$ of 0.5-0.8.
   b. Induce with 1mM IPTG for 4hr at 37°C.
   c. Pellet cells by centrifugation and re-suspend in 2ml TNG buffer.
   d. Sonicate to disrupt bacterial cell walls to release soluble proteins.

2. *Optional*:
   a. Purify *soluble* S11 fusion protein by metal-affinity column.
   b. Determine the purity and quantify protein.

3. Store the fusion proteins at -20°C until *In vitro* complementation assay is performed.

E. *In Vitro* Complementation assay

1. Prepare 96-well plate(s)
   a. Block 96-well flat bottom microplate with 0.5% w/v bovine serum albumin in TNG, 10 min.
   b. Serially dilute positive control reagent 22μl:22μl in TNG buffer from 5μM-39nM, or lower depending on instrument sensitivity.
   c. Add 20μl of each standard dilution to the first two columns of a 96-well plate (see diagram).
   d. Add 20μl aliquots of the protein fusion(s) prepared in part C. to remaining wells in the same 96-well plate.
   e. Prepare a negative control of 0.5% BSA in TNG buffer and add 20ul to the plate.
   f. Perform complementation by adding 180μl aliquot of GFP 1-10 to all of the above wells except the blank.
2. Monitor fluorescence kinetics with a microplate fluorescence reader or fluorimeter (λ_{exc}=488nm/ λ_{em}=530nm), at 3min intervals for 15h.

DATA ANALYSIS

Data Analysis

Subtract the blank fluorescence values from the final fluorescence values of the sample(s) and the positive control. Estimate protein concentration by comparing fluorescence on the standard curve.

REFERENCES

1. Waldo et al. "Rapid protein-folding assay using green fluorescent protein," *Nature Biotechnology* 17, 691 - 695, July 1999.
2. Cabantous et al. "Protein tagging and detection with engineered self-assembling fragments of green fluorescent protein," *Nature Biotechnology* **23,** 102-107, December 2004.

Suggested Plate Configuration

	1	2	3	4	5	6	7	8	9	10	11	12
A	**Neat positive control**	**Neat positive control**	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown
B			Unknown	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown
C			Unknown	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown
D			Unknown	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown
E			Unknown	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown
F			Unknown	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown
G	▼	▼	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown	**Blank**	**Blank**
H	**.39nM positive control**	**.39nM positive control**	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown	**BSA negative control**	**BSA negative control**

For Technical Support and Customer Service: (505) 342-0224 / (866) 342-0224
Sandia BioTech, Inc. • 609 Broadway, NE • Albuquerque • New Mexico• 87102
www.SandiaBio.com

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