3050 Spruce Street, St. Louis, MO 63103 USA
Tel: (800) 521-8956 (314) 771-5765 Fax: (800) 325-5052 (314) 771-5757
email: techservice@sial.com sigma-aldrich.com

Product Information

***In Vitro* Bacterial Split Fluorescent Protein "Fold 'n' Glow"™ Solubility Assay Kits**

Catalog Numbers
APPA001 – *In Vitro* Bacterial Split GFP "Fold 'n' Glow" Solubility Assay Kit (Green)
APPA008 – *In Vitro* Bacterial Split CFP "Fold 'n' Glow" Solubility Assay Kit (Cyan)
APPA009 – *In Vitro* Bacterial Split YFP "Fold 'n' Glow" Solubility Assay Kit (Yellow)
Storage Temperature –20 °C

TECHNICAL BULLETIN

Product Description

The "Fold 'n' Glow"™ Protein Solubility Assay kit allows a test protein to be expressed as an N-terminal fusion with a fluorescent protein [i.e., Green Fluorescent Protein (GFP), Cyan Fluorescent Protein (CFP), and Yellow Fluorescent Protein (YFP)]. This allows the detection of protein 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.

Fluorescent protein (GFP, CFP, or YFP) fusions and split protein tags are widely used for the analysis of protein. These large tags can perturb protein solubility, misfold, and alter the processing of the protein. The split fluorescent protein technology used in the "Fold 'n' Glow" assay overcomes these problems. The protein tag is a genetically encoded, split fluorescent protein technology, engineered with small, soluble, self-associating fragments. Thus, it is a simple split fluorescent protein 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 fluorescent protein technology. The fluorescent protein system is a simple and easy tagging and detection system.[2] These kits 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 kit is a protein tagging and detection method that uses split fluorescent protein technology in a fluorescent complementation assay. The protein to be quantified is fused to a small fluorescent protein fragment (contained in the S11 plasmid) via a flexible linker. Expressed separately, neither the fusion protein of interest nor the fluorescent protein detector (Universal Detection Reagent S1-10) is fluorescent. When mixed, the properly folded fusion protein and detector spontaneously associate, completing the fluorophore. Misfolding or aggregation of the fusion protein makes the fluorescent protein tag inaccessible and prevents complementation, thus preventing fluorescence. Therefore, misfolded or aggregated proteins are not included in the quantification of the protein of interest.

Components

The kit contains sufficient reagents for one 96 well plate (96 tests).

Reagent	Amount
Bacterial S11 Plasmid (APPA004)	1 vial
Positive Control (APPA003)	1 vial
Universal Detection Reagent (S1-10): Green (APPA001) or Cyan (APPA008) or Yellow (APPA009)	20 mL

Universal Detection Reagent (S1-10) – Complementary fluorescent protein fragment. Supplied ready to use.

Positive Control (APPA003) – Positive control fluorescent protein fusion protein. Supplied ready to use at 5 μM.

Bacterial S11 Plasmid (APPA004) – Kanamycin resistance (Kan^R) with *Bam*H I and *Nde* I restriction sites. Supplied at 100 ng/mL. For additional restriction sites and plasmid information, see Appendix A.

Reagents and Equipment Required but Not Provided.

- GC5™ competent cells (Cat. No. G3169)
- Kanamycin
- IPTG (Ispropyl β-D-thiogalactopyranoside)
- LB growth media and plates
- *Bam*H I and *Nde* I restriction enzymes
- Ligation materials
- Metal affinity column
- TNG Buffer (50 mM Tris, pH 7.4, 0.1 M NaCl, and 10% glycerol)
- Bovine serum albumin (BSA)
- Plasmid isolation reagents
- 96 well plate
- Incubator
- Centrifuge
- Sonicator
- Microplate fluorescence reader
- Vortex mixer
- Water bath

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

The components of this kit remain active for approximately 6 months when stored at –20 °C.

Depending on the particular usage requirements, it may be appropriate to re-aliquot reagents to smaller working volumes to avoid repeated freeze-thaw cycles or repeated pipetting from the same vial.

Procedures

A. Preparation of insert DNA
   1. Perform plasmid prep and/or PCR.
   2. Use *Nde* I (5′) and *Bam*H I (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 a renewable source of plasmid DNA, transform the plasmid vector provided in this kit in an *E. coli* host strain. It is recommended bacterial frozen stocks be prepared for all transformed plasmids using standard molecular biology techniques.
   2. Purify plasmid DNA for cloning using a plasmid purification kit or other plasmid isolation technique.
   3. Perform restriction enzyme digest of the S11 vector using *Nde* I and *Bam*H I to prepare the plasmid for inserting DNA.
      Optional: Dephosphorylate the digest to decrease the non-recombinant background. Use molecular biology grade calf intestinal or shrimp alkaline phosphatase according to the manufacturer's directions.
   4. Perform ligation reaction according to manufacturer's instructions.
   5. Store vector at –20 °C until ready to use.

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 S11 fusion plasmid.
   2. Transform the S11 fusion plasmid in an expression host for high yields of quality plasmid. Use standard methods 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 200 mL culture of bacteria, transformed S11 fusion protein, in LB growth medium with Kanamycin (20–50 μg/mL) to log phase at $OD_{600\ nm}$ of 0.5–0.8.
      b. Induce with 1 mM IPTG for 4 hours at 37 °C.
      c. Pellet the cells by centrifugation and re-suspend in 2 mL of TNG buffer.
      d. Sonicate to disrupt the bacterial cell walls and release soluble protein.

      Optional: Purify *soluble* S11 fusion protein by metal-affinity column. Determine the purity and quantity of protein.
   2. Store the S11 fusion protein at –20 °C until *in vitro* complementation assay is performed.

E. *In vitro* Complementation assay
   1. Prepare 96 well plate(s).
      a. Block a 96 well flat bottom microplate for 10 minutes with 0.5% (w/v) bovine serum albumin in TNG buffer.
      b. Prepare 7 serial dilutions of Positive Control (SRS11, 5 μM) with TNG buffer (22 μL:22 μL) down to 39 nM, or lower depending on instrument sensitivity.
      c. Add 20 μL of the Positive Control (SRS11, 5 μM) and each serial dilution to the appropriate wells of the first two columns of a 96 well plate (see Appendix B).
      d. Add 20 μL aliquots of the protein fusion(s) prepared in Procedure, part C to the remaining wells in the same 96 well plate.
      e. Prepare a negative control of 0.5% BSA in TNG buffer and add 20 μL to the plate.
      f. Perform complementation by adding 180 μL aliquot of Universal Detection Reagent (S1-10, i.e., GFP, CFP, or YFP) to all the wells, except the blank.
   2. Monitor the fluorescence kinetics with a microplate fluorescence reader or fluorimeter at 3 minute intervals for 15 hours. Use the excitation and emission wavelengths in Table 1 for monitoring fluorescence.

Table 1.
Fluorescence wavelengths

Fluorescent protein	$\lambda_{excitation}$	$\lambda_{emission}$
GFP	488 nm	525 nm
CFP	488 nm	500 nm
YFP	488 nm	550 nm

Note: Emission wavelengths can be ±25 nm

Results

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, G.S. et al., Rapid protein-folding assay using green fluorescent protein. Nature Biotechnology, **17**, 691-695 (1999).
2. Cabantous, S. et al., Protein tagging and detection with engineered self-assembling fragments of green fluorescent protein. Nature Biotechnology, **23**, 102-107 (2004).
3. Cabantous, S., and Waldo, G.S., *In vivo* and *in vitro* protein solubility assays using split GFP. Nature Methods, **3**, 845-854 (2006).

Appendix A
Plasmid (S11)

1. *Nco* I site CCATGG has initiator methionine
2. 6HIS tag CATCATCATCATCATCAC amino acids HHHHHH
3. Trombin site amino acids SGLVPPRGS
4. Cloning site *Nde* I CATATG
5. Frame shift stuffer TAATTAATTAATT
   Note: This is a +1 frame shift to make sure S11 doesn't express unless in-frame construct between *Nde* I/*Spe* I or *Nde* I/*Bam*H I. Typically, a user can digest *Nde* I + *Spe* I and clone that way, or *Nde* I + *Bam*H I and clone that way. See reference 3 for guidelines on primer design.
6. *Spe* I ACTAGT (expressed as amino acids TS)
7. *Bam*H I GGATCC (expressed as amino acids GS)
8. Linker amino acids DGGSGGGSTS
9. Fluorescent Protein S11 tag
10. Stop codon

Appendix B
Suggested 96 well 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	**.39 nM positive control**	**.39 nM positive control**	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown	**BSA negative control**	**BSA negative control**