assay designs
SIMPLIFY YOUR SCIENCE

> Methyltransferase Activity Kit

Catalog # 907-025

Sufficient Reagents for 96 tests
For use with purified *in vitro* samples

Reagents require separate storage conditions.

Check our website for additional protocols, technical notes and FAQs.

For proper performance, use the insert provided with each individual kit received.

Table of Contents

Patent Pending

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

Assay Designs, Inc.
734.668.6113 or 800.833.8651
www.assaydesigns.com

Introduction

Assay Designs' Methyltransferase Activity kit is a complete kit for the screening of candidate compounds that may alter normal methyltransferase activity. Please read the complete kit insert before performing this assay.

Methylation of proteins, nucleic acids and oligosaccharides is an important post-translational regulatory event. Activities that are methylation-related include meiosis, biosynthesis, development, signal transduction, chromatin regulation, and gene silencing.[1-9] The enzymes that mediate the covalent transfer of a methyl group from a donor to an acceptor molecule are methyltransferases. Methyltransferases have structurally unrelated acceptors as diverse as proteins and DNA, however frequently use S-adenosyl-L-Methionine as a universal donor.[10] Part of the acceptor diversity of this enzyme family relates to the flexible structural folds that bind these molecules in proximity of the donor.[11] The side-chains of lysine, arginine, glutamate, glutamine, asparagines, and isoprenylated residues serve as methylation sites in proteins like histones.[1] Changes in methylation patterns have been tightly linked to disease states such as cancer and vascular disease.[12-14]

Principle

1. Enzyme, reaction mix spiked with acceptor substrate and detection solution are added to wells of a black 96-well plate. The plate is then incubated.

2. Ice cold isopropyl alcohol is added to stop the reaction.

3. The plate is transferred to a plate reader and fluorescence is measured at 380ex/520em.

Materials Supplied

1. Black Microtiter Plate
   One plate of 96 wells, Catalog No. 80-1675
   The plate is ready to use.

2. Transferase Assay Buffer Concentrate
   15 mL, Catalog No. 80-1648
   A 10X concentrated buffer containing detergent and preservative.

3. Methyltransferase Reaction Buffer Concentrate
   0.3 mL, Catalog No. 80-1649
   A 10X concentrated buffer containing S-adenosyl-L-methionine.

4. Transferase Detection Solution Concentrate.
   0.15 mL, Catalog No. 80-1650
   A 100X concentrated solution of fluorescent substrate in DMSO.

5. Methyltransferase Positive Control
   0.2 mL, Catalog No. 80-1652
   A Stock Solution of buffer and reaction end product.

6. Foil Plate Sealer
   3 each, Catalog No. 10-3126

7. Methyltransferase Assay Layout Sheet
   1 each, Catalog No. 30-0241

Do not mix components from different kit lots or use reagents beyond the expiration date of the kit.

The physical, chemical, and toxicological properties of the chemicals and reagents contained in this kit may not have been fully investigated. Therefore, we recommend the use of gloves, lab coats, and eye protection while using any of these chemical reagents.

Detection Solution contains DMSO. Avoid skin contact. Avoid breathing vapor.

Assay Designs
734.668.6113 or 800.833.8651
www.assaydesigns.com

Storage

Reagents require separate storage conditions.

All kit components are stable at their recommended storage temperatures until the kit expiration date. The recommended storage temperature does not necessarily reflect shipping conditions.

Materials Needed but Not Supplied

1. Deionized or distilled water.
2. S-adenosyl-L-methionine dependent methyltransferase.
3. Appropriate methyltransferase acceptor substrate.
4. Inhibitor/activator compounds to be screened.
5. Precision pipets for volumes between 5 µL and 1,000 µL.
6. Disposable beakers for diluting buffer concentrates.
7. Graduated cylinders.
8. A microplate shaker.
9. Disposable microtubes, 0.5 and 1.5 mL.
10. Microplate reader capable of measuring fluorescence at 380ex/520em.
11. Crushed ice and container.
12. Isopropyl alcohol (ice cold).

Reagent Preparation

1. **Transferase Assay Buffer**
   Prepare the assay buffer by diluting 15mL of the supplied Transferase Assay Buffer Concentrate with 135mL of deionized water. This solution can be stored at 4°C for 3 months, or the kit's expiration, whichever is earlier. The 1X assay buffer is used to prepare dilutions of Methyltransferase Reaction Mix, Transferase Detection Solution, enzymes, substrates, and compounds to be screened.

2. **Transferase Detection Solution**
   Count the total number of wells needed for compound screening and add 6 (for the zero, positive control, and blank wells in duplicate). Use the following formula to calculate the volume of 1X Detection Solution required.

   A. Total volume required
   [Total number of wells needed + 6] x 100µL = _____ µL

   B. Volume of Transferase Detection Solution Concentrate required
   [Total volume required (from A. above)] x 0.01 = _____µL

   C. Volume of 1X Transferase Assay Buffer required
   [Total volume required (from A. above)] x 0.99 = _____ µL

   Prepare 1X Detection Solution by combining the appropriate reagent volumes calculated in B and C above. For example, to prepare 2 mL of 1X Detection Solution, combine the following volumes: 20µL of the supplied Transferase Detection Solution Concentrate and 1980 µL 1X Transferase Assay Buffer. Diluted Detection Solution should be kept on ice and used within 8 hours of preparation. Any unused 1X Detection Solution should be discarded.

3. **Methyltransferase Reaction Mix**
   Count the total number of wells needed for compound screening and add 6 (for the zero, positive control, and blank wells in duplicate). Use the following formula to calculate the volume of 1X Methyltransferase Reaction Mix required.

   A. Total volume required
   [Total number of wells needed + 6] x 25µL = _____ µL

   B. Volume of Methyltransferase Reaction Buffer Concentrate required
   [Total volume required (from A. above)] x 0.1 = _____µL

   C. Volume of acceptor substrate required
   [Determined empirically based on enzyme used] = _____µL

Thaw Detection Solution Concentrate at room temperature. Do not place on ice. Thaw all other reagents on ice.

Pre-rinse each pipet tip with reagent.

Store1X Detection Solution on ice and use within 8 hours of preparation. Discard unused 1X Solution.

A minimum of 2 wells of the positive control are recommended. Adjust the calculations accordingly if more than 2 wells are used.

Detection Solution contains DMSO. Avoid skin contact. Avoid breathing vapor.

Store1X Reaction mix on ice and use within 8 hours of preparation. Discard unused 1X Solution.

Assay Designs
734.668.6113 or 800.833.8651
www.assaydesigns.com

D. Volume of 1X Transferase Assay Buffer required
[Total volume required (from A. above)] x 0.90 – [volume of substrate required] = _____ µL

Prepare 1X Reaction Mix on ice by combining the appropriate reagent volumes calculated in B, C and D above. For example, to prepare 2 mL of Reaction Mix spiked with 22µL of acceptor substrate, combine the following volumes: 200µL Methyltransferase Reaction Buffer Concentrate, 22µL substrate and 1778µL 1X Transferase Assay Buffer. Diluted Reaction Mix should be kept on ice and used within 8 hours of preparation. Any unused 1X Reaction Mix should be discarded.

Thaw Methyltransferase Positive Control on ice.

4. **Positive Control**
A positive control is included in the kit to verify the activity of the kit components. It should not be used to calculate the concentration of methyltransferase activity in samples.

Prepare enough Positive Control to test in duplicate. Use the following formula to calculate the volume of Positive Control required. A minmum of 2 wells of the positive control are recommended per assay.

A. Total volume required
[Total number of wells needed] x 25µL = _____µL

B. Volume of Positive Control required
[Total volume required (from A. above)] x 0.1 = _____µL

C. Volume of 1X Transferase Assay Buffer required
[Total volume required (from A. above)] x 0.90 =_____µL

Store Dilute Positive Control on ice and use within 8 hours of preparation. Discard unused 1X solution.

Prepare Positive Control on ice by combining the appropriate reagent volumes calculated in B and C above. For example, to prepare 200µL of Positive Control, combine the following volumes: 20µL of Positive Control and 180µL of 1X Transferase Assay Buffer. Diluted Positive Control should be kept on ice and used within 8 hours of preparation. Any unused 1X Positive Control should be discarded.

Sample Handling

This assay is suitable for use with all S-adenosyl-L-methionine dependent methyltransferases. It is necessary to titrate each enzyme/substrate system in the assay to determine optimal conditions.

This assay should only be used to screen purified *in vitro* samples in buffer systems without reductants.

It is recommended that an end-point assay be performed to determine the optimal concentration of enzyme/substrate to use prior to screening candidate compounds. Make serial dilutions of the methyltransferase of interest in the assay buffer. Initial concentrations of 100 nM are recommended. A kinetic assay format is also an available option.

The positive control provided may also be used to test colored compounds for interference in the assay. A suitable protocol follows.

If buffers other than those provided are used in the assay, the end-user must determine the appropriate dilution and assay validation.

Before screening colored compounds, the compound should be titered in the assay to ensure that there is no interference with signal collection.

End point / Kinetic Assay Procedure

Refer to the Assay Layout Sheet to determine the number of wells to be used. Cover unused wells tightly with a plate sealer. **DO NOT REUSE WELLS!**

1. Pipet 25 µL of 1X Transferase Assay Buffer into the blank wells.
2. Pipet 25 µL of methyltransferase dilutions into the bottom of the appropriate wells.
3. Pipet 25 µL of 1X Reaction Mix into each well.
4. Pipet 100 µL 1X Detection Solution into each well.
5. Cover plate with foil plate sealer. Incubate for 20 min (for end point format) shaking* at room temperature.
   For **Kinetic** measurements, incubate identical reaction wells for the desired periods of time. The duplicate wells for each time point can be stopped as in step #10 below.
6. Pipet 25 µL of 1X Positive Control into the bottom of the appropriate wells.
7. Pipet 25 µL of 1X Reaction Mix into Positive Control wells.
8. Pipet 100 µL Detection Solution into Positive Control wells.
9. Cover plate with foil plate sealer. Incubate for 10 min.
10. Pipet 50 µL of ice cold isopropyl alchol into each well.
11. Read fluorescence at 380ex/520em.

* Shaking is preferably carried out on a suitable plate or orbital shaker set at a speed to ensure adequate mixing of the contents of the wells. The optimal speed for each shaker will vary and may range from 120-700 rpm.

All samples should be run in duplicate.

Pre-rinse each pipet tip with reagent. Use fresh pipet tips for each sample and reagent.

Pipet the reagents to the sides of the wells to avoid possible contamination.

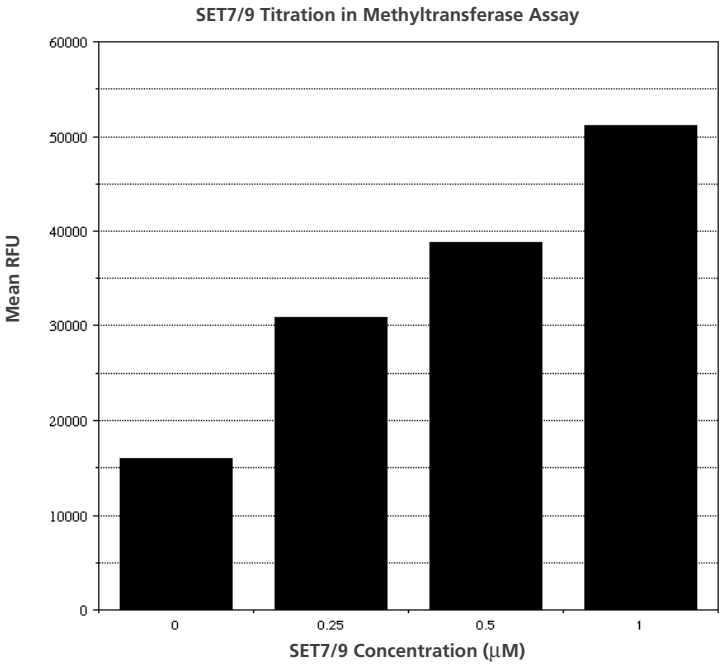
Assay Designs
734.668.6113 or 800.833.8651
www.assaydesigns.com

Example of End point Assay Results

1. Plot the mean of the duplicate relative fluorescence units (RFU) at 380ex/520em versus Enzyme concentration.

2. Calculate the signal to noise ratio : $\frac{\text{mean RFU for enzyme dilution}}{\text{mean RFU blank}}$.

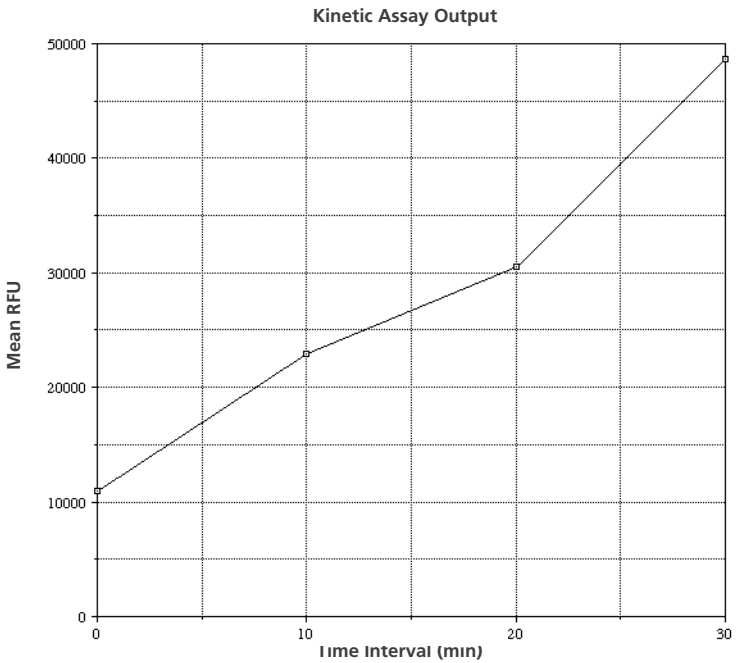
An engineered portion of the human lysine specific histone methyltransferase, SET7/9 was titered in the assay using 15µM of the peptide substrate TAF-10. Serial dilutions of the enzyme were prepared in Transferase Assay Buffer. Average relative fluorescence was plotted against SET7/9 concentration to generate the following graph. This is for illustration purposes only. The investigator must titrate their own enzyme substrate system in the assay.



Based on this titration data, the methyltransferase concentration of 0.5µM produces a maximum signal within the detection range of the plate reader, with a signal to noise ratio sufficient for easy detection of altered enzyme activity.

Example of Kinetic Assay Results:

0.5μM SET7/9 was tested with 15μM TAF-10 substrate in the kinetic assay format. Mean relative fluorescence was plotted against the stop time interval to generate this graph. This graph is for illustration purposes only. The kinetic assay must be optimized by the investigator, with their enzyme / substrate system.



Assay Designs
734.668.6113 or 800.833.8651
www.assaydesigns.com

Typical Inhibition Assay Procedure

Refer to the Assay Layout Sheet to determine the number of wells to be used. Cover unused wells tightly with a plate sealer. **DO NOT REUSE WELLS!**

1. Pipet 25µL of 1X Transferase Assay Buffer into the blank wells.
2. Pipet 10µL of 1X Transferase Assay Buffer into the zero wells.
3. Pipet 10µL of inhibitor dilution into the bottom of the appropriate wells.
4. Pipet 15µL of methyltransferase at chosen working concentration into appropriate wells.
5. Cover plate with foil plate sealer. Incubate for 10 min at room temperature without shaking.
6. Pipet 25µL of 1X Reaction Mix into each well.
7. Pipet 100µL of 1X Detection Solution into each well.
8. Cover plate with foil plate sealer. Incubate for 30 min, shaking* at room temperature.
9. Pipet 50µL of ice cold isopropyl alcohol into each well.
10. Read fluorescence at 380ex/520em.

* Shaking is preferably carried out on a suitable plate or orbital shaker set at a speed to ensure adequate mixing of the contents of the wells. The optimal speed for each shaker will vary and may range from 120-700 rpm.

Calculation of Results

Several options are available for the calculation of the inhibition of methyltransferase. We recommend that the data be handled by a software package utilizing a suitable curve fitting program to determine the percent inhibition. If data reduction software is not readily available, the data can be calculated as follows:

1. Calculate the mean net RFU for each sample by subtracting the mean blank RFU from the mean RFU for the samples:

   Mean Net RFU = Mean Sample RFU - Mean Blank RFU

2. Percent inhibition should be calculated using the following formula for each inhibitor dilution:

Percent Inhibition =

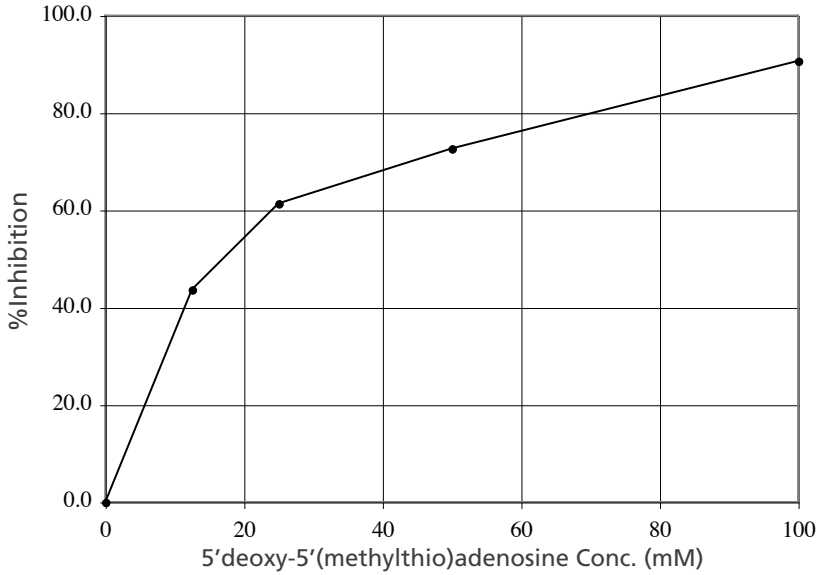
$$\frac{(\text{Mean Net Zero (non-inhibited enzyme) RFU} - \text{Mean Net Inhibited enzyme RFU})}{\text{Mean Net Zero (non-inhibited enzyme) RFU}} \times 100$$

Typical Inhibition Assay Results

Using SET7/9 and TAF-10 as an enzyme substrate system, percent inhibition for dilutions of 5'deoxy-5'(methylthio)-adenosine was tested. The results shown below are for illustration only and **should not** be used to calculate results from another assay.

Dilution	Inhib. Conc. (mM)	Mean RFU	Mean Net RFU	% Inhibition
1	100 mM	12828	1135	90.7
2	50 mM	15030	3337	72.7
3	25 mM	16411	4718	61.4
4	12.5 mM	18575	6882	43.7
0	0 mM	23910	12217	0
blank	---------	11693	0	------

Typical Inhibition Curve



Assay Designs
734.668.6113 or 800.833.8651
www.assaydesigns.com

Typical Enzyme Interference Assay Procedure

1. Pipet 25µL of 1X Transferase Assay Buffer into the blank wells.
2. Pipet 10µL of 1X Transferase Assay Buffer into the zero wells.
3. Pipet 10µL of colored compound dilution into appropriate wells
4. Pipet 15µL of 1X Positive Control into the zero wells, and wells containing colored compound dilutions.
5. Pipet 25µL of 1X unspiked reaction mix into each well.
6. Pipet 100µL 1X Detection Solution into each well.
7. Cover plate with foil plate sealer, incubate for 10 min at room temperature without shaking.
8. Pipet 50µL of ice cold isopropyl alcohol into each well.
9. Read fluorescence at 380ex/520em.

To determine whether or not colored compounds will interfere with the assay, calculate and compare the signal to noise ratio of the colored compound dilutions to the signal to noise ratio of the zero wells.

Interfering Substances

The following solvents were tested for interference with the fluorescent signal generated in the assay. The table lists the percentage of signal in the presence of interferant relative to the zero for each solvent.

% Interferant	DMSO	DMF	Acetonitrile
12.5	108	68	49
6.25	107	82	71
3.12	107	88	86
1.56	112	98	96
0.78	114	104	95
0.39	115	109	100
0	100	100	100

* Percent interferant is relative to a 50 µL total reaction volume.

Diluents containing bovine serum albumin (BSA), or other Thiol containing reagents, should be treated with N-Ethylmaleimide (NEM) prior to use in the assay. We recommend reacting 10% BSA with 1 mM NEM for 1 hour at room temperature, however this procedure should be optimized for each reagent.

References

1. Walsh, C.T. Protein Methylation in Postransaltional Modifications of Proteins, Expanding Nature's Inventory. Roberts and Co. Pub.; Englewood, USA.
2. Hayashi, K. et. al. A Histone H3 Methyltransferase Controls Epigenetic Events Required for Meiotic Prophase. Nature (2005) 438(17):374-378.
3. Mavrodi, D.V. et. al. Functional Analysis of Genes for Biosynthesis of Pyocyanin and Phenazine-1-Carboxamide from Pseudomonas aeruginosa PAO1. J. Bact. (2001) 183(21):6454-6465.
4. Chubb, J.R. et. al. Developmental Timing in Dictyostelium is Regulated by the SET1 Histone Methyltransferase. Devel. Bio. (2006) 292:519-532.
5. Miao, F. et. al. Coactivator-Associated Arginine mthyltransferase-1 Enhances Nuclear Factor-kB-Mediated Gene Transcription Thought Methyaltion of Histone H3 at Arginine 17. Mol. Endocrin. (2006) 20(7):1562-1573.
6. Ehrenhofer-Murray, A.E. Chrmatin Dynamics at DNA Replication, Transcription and Repair. Eur. J. Biochem. (2004) 271:2335-2349.
7. Kouskouti, A., et. al. Gene-Specific Modulation of TAF10 Function by SET9-Mediated Methylation. Mol. Cell (2004) 14:175-182.
8. Chulkov, S. et. al. Regulation of p53 Activity Through Lysine Methylation. Nature (2004) publ on-line (doi:10.1038/nature03117).
9. Fuks, F. et. al; The DNA Methyltransferases Associate with HP1 and the SUV399H1 Histone Methyltransferase. Nucl. Acids Res. (2003) 31(9):2305-2312
10. Chaing, P.K., et. al. S-Adenosylmethionine and Methylation. FASEB J. (1996) 10:471-480.
11. Trievel, R.C. Structure and Function of Histone Methyltransferases. Critical ReviewTM in Eukaryotic Gene Expression (2004) 14(3):147-169.
12. Robertson, K.D. DNA Methylation and Human Disease. Nature Rev. (2005) DNA Methylation Collection:7-20.
13. Wei, S.H. et.al. Methylation Microarray Analysis of Late-Stage Ovarian Carcinomas Distinguishes Progression-free Survival in Patients and Identifies Candidate Epigenetic Markers. Clin. Cancer Res. (2002) 8:2246-2252.
14. Castro, R. et. al. Increased Homocysteine and S-Adenosylhomocysteine Concentrations and DNA Hypomethylation in Vascular Disease. Clin. Chem. (2003) 49(8):1292-1296.

Assay Designs
734.668.6113 or 800.833.8651
www.assaydesigns.com

Assay Designs
734.668.6113 or 800.833.8651
www.assaydesigns.com

Limited Warranty

Assay Designs, Inc. warrants that at the time of shipment this product is free from defects in materials and workmanship. This warranty is in lieu of any other warranty expressed or implied, including but not limited to, any implied warranty of merchantability or fitness for a particular purpose.

MSDS (Material Safety Data Sheet) available online

Assay Designs must be notified of any breach of this warranty within 48 hours of receipt of the product. No claim shall be honored if Assay Designs is not notified within this time period, or if the product has been stored in any way other than outlined in this publication. The sole and exclusive remedy of the customer for any liability based upon this warranty is limited to the replacement of the product, or refund of the invoice price of the goods.

Assay Designs, Inc.
5777 Hines Drive
Ann Arbor, Michigan
USA

tel: 734.668.6113 or 800.833.8651
fax: 734.668.2793
info@assaydesigns.com
www.assaydesigns.com

technical support: technical@assaydesigns.com

Kits for Charity™

Assay Designs has always been an active contributor to a number of local, national and international charities. We have broadened our charitable contributions by implementing a program called Kits for Charity™.

Each quarter, Assay Designs will feature a different non-religious and non-political charitable organization on our website. For each kit sold during this time period, we will make a monetary contribution to the featured charity. Please check our website for the current quarter's charity to see what organization your purchases are helping to support.

If you have any suggestions for future Kits for Charity™ recipients, please contact us at 800.833.8651 or 734.668.6113.

Contact Us

For more details concerning the information within this kit insert, or to order any of the Assay Designs' products, please call (734) 668-6113 between 8:30 a.m. and 5:30 p.m. EST. Orders or technical questions can also be transmitted by fax or e-mail 24 hours a day.

Catalog No. 25-0600

Last Revised: May 8, 2007