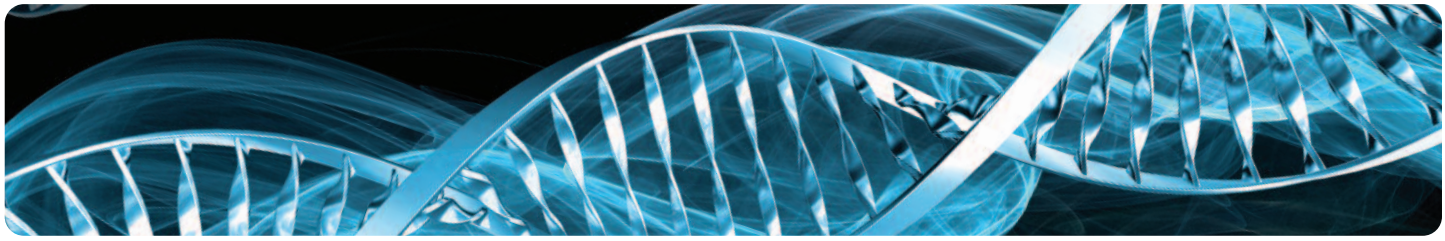
Honeywell

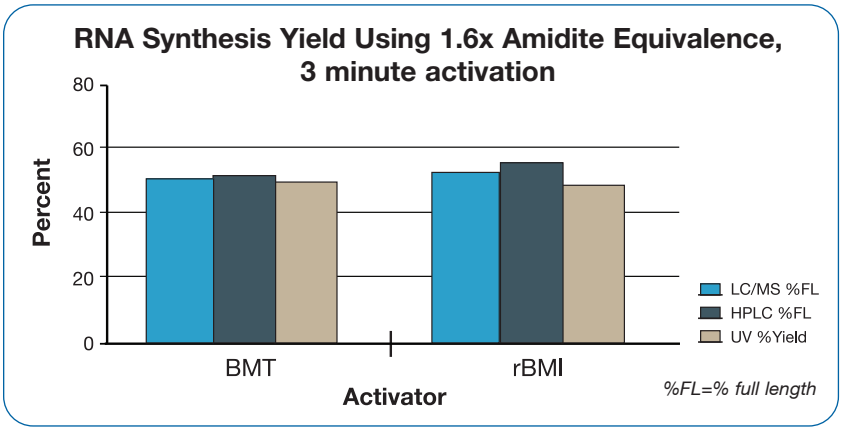
Comparison Between rBMI and BMT Activator Reagents

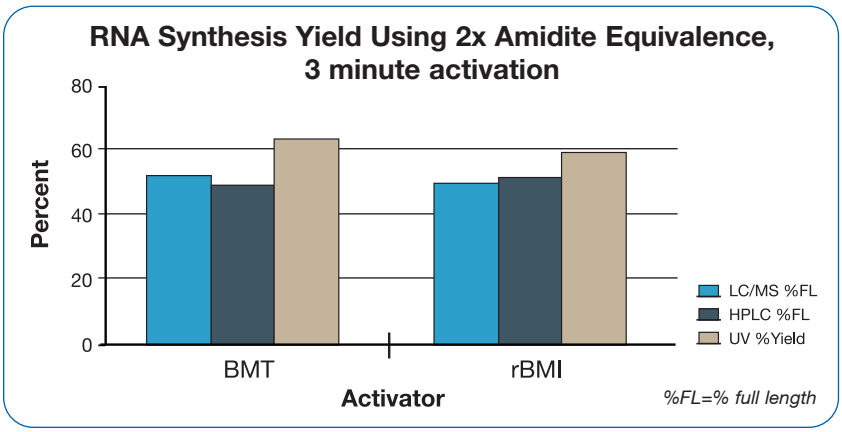


Honeywell Burdick & Jackson® rBMI activator is a patented* coupling reagent that is composed of 5-Benzylmercaptotetrazole (BMT) and N-Methylimidazole (NMI) in acetonitrile. In the industry, 0.3M BMT is generally accepted as an activator reagent for RNA synthesis. Honeywell Burdick and Jackson adds NMI to the BMT activator to increase the solubility of BMT in acetonitrile.

Temperature and Solubility Comparison

The solubility limit of BMT in acetonitrile is 0.44M. The addition of NMI as a co-solvent increases the solubility limit of BMT above 0.5M. It also allows the BMT to stay in solution at colder temperatures. This is an important benefit, especially when shipping a solution of BMT in acetonitrile during the colder winter months. rBMI will stay in solution even at temperatures lower than -12°C (10°F), whereas a solution of 0.3M BMT in acetonitrile will begin to precipitate at 5°C (41°F). When transporting and storing rBMI during colder periods, it may be possible to avoid resolubilizing the BMT in the acetonitrile which may otherwise be required if a reagent does not contain the NMI co-solvent.





Comparison of Activation Efficiency

We studied the effect of NMI on the activation ability of BMT in acetonitrile solution to determine whether the NMI negatively impacts the synthesis process. Specifically, we compared the results of synthesizing RNA using 0.3M BMT in acetonitrile to those results using a solution of rBMI, which consists of 0.3M BMT and 0.5% NMI in acetonitrile. RNA yield was measured using three methods, LC/MS, HPLC and UV, to fully assess the potential differences between both activators. *The charts to the left show the experimental results from synthesizing a 20-mer sequence under differing activator reagent and amidite conditions.*

Our results indicate that adding NMI to the BMT activator does not impact the synthesis yield of RNA. This result was confirmed using both 1.6x and 2x excess amidites.

For a full list of experimental parameters, please see the reverse side.

This experimental work is based on the following parameters:

- RNA sequence: 5’ cga ucu ucu gga aau cca aT 3’
- Primer support: GE Healthcare PS200 T80s (polystyrene beads, 80 µmole/g)
- Amidites: 2’-tBDMS protected: A (n-bz), C (n-acetyl), G (n-acetyl), U
- Amidite excess: 1.6x or 2x
- Synthesizer: ÄKTA™ oligopilot™ plus 100
- Synthesis scale: 100 µmole
- Coupling time: 3 minutes
- Analytical instrumentation:
  - LC/MS: Agilent 6210 Time-of-Flight (TOF) with Agilent Eclipse plus C18, 3.5 micron X 5 cm column, ESI negative mode
  - HPLC: Agilent 1100 with Dionex DNAPac 200 column
- Honeywell Burdick & Jackson BioSyn® reagents:
  - Deblock Reagent, cat. no. SR622 (3% Dichloroacetic acid in Dichloromethane (v/v))
  - Activator Reagent: rBMI Activator, cat. no. BR731RN (0.30M 5-Benzylthio-1H-tetrazole, 0.5% NMI, 99.5% Acetonitrile)
  - Capping Reagent Cap A**, cat. no. SR644 (20% Acetic Anhydride, 30% 2,6-Lutidine, 50% Acetonitrile (v/v/v))
  - Capping Reagent Cap B†, cat. no. BR654 (20% N-Methylimidazole, 80% Acetonitrile (v/v))
  - Oxidation Reagent, cat. No. BR664 (0.05M Iodine, 10% Water, 90% Pyridine (v/v))

Percent yield definitions:

- HPLC % FL (full length): The percent of the main peak as determined by the UV absorbance at 260nm
- LC/MS % FL (full length): The percent (as determined by the UV absorbance at 260nm) of the full-length oligomer with the correct mass as determined by deconvolution of the extracted ions at the retention time of the main peak
- UV yield: the ratio of actual amount of RNA made over the theoretical RNA amount that could be made with the primer support, based on UV absorption at 260nm

For additional information or to request a sample, visit www.honeywell.com/contactbandj

* U.S. Patent No. 7,339,052
**GE Healthcare product designation for this formulation: Capping B
†GE Healthcare product designation for this formulation: Capping A

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