DC DIAGNOCINE

DM270	DIG Labeled Blue Color Marker for Small RNA

Q1: **I am using DIG Labeled Blue Color Marker for Small RNA (cat no: DM270). Just want to know the approximate concentration of each RNA in the marker. If I load 5 ul of this marker, can I get 1 pmol for each band?**

A1: First, we assume that you are requesting information for RNA marker as in single strand RNA that we use in marker mix. Below comments are relevant to ssRNA band.

As you know, each band (lowest size to largest in marker prep) concentration is different so that each band shows on the gel at about equal intensity for visual purposes. For example, if the lowest band is 50base pair, then the loading concentration would be in range of 50-200ng and the larger ones would be less when you load 5ul per lane. The larger the size, the less concentration we need to visualize in the gel. The smaller the size, the more concentration of the band (RNA) is needed in order to visualize. In all cases, we have enough concentration of each band to visualize when you load 5ul in the gel. If you wish to visualize stronger marker bands, you have to load more. In Japan, the lot to lot variance is minimal, so you will get consistent result if the molarity of the band if very important to your experiment.

If we consider smaller size (i.e. 50base pair) band, it would only be in pico molar range; this is assuming that you load 5ul of the marker mix. We expect >20ng of the lowest size band and it would be over 1 pico molar range and that meets your request.

We do not know the purpose of your minimal 1pmol range for the marker bands, but our marker mix should meet your requirement of minimum 1 pico molar range for the lowest size band as in ssRNA. The conc of larger band would increase but all bands should be in 1pico molar range.

Q2: **I recently purchased the DynaMarker DIG labeled blue color marker for small RNA from you and have now used it for Northern Blotting. I loaded the marker according to instructions and the blue color bands show up on the nylon membrane after electroblotting. However, while my own probes give strong results upon detection with AP-coupled anti-DIG antibody, the marker does not show any DIG signal, only the blue bands.**

A2: We understand that your method is chromogenic development. We see that your markers are clearly shown on the marker lane and not showing chromogenic activity.

The protocol and the markers were developed for the chemiluminescent substrate. In some cases, the customers load a lot of makers, so we occasionally suggest serial dilution just in case they are burning out the substrate.

In your case, you are using the chromogenic development but it should theoretically work. However, the signal is not there.
This appears that the sensitivity to detect DIG from the marker is not enough. If you see the figure below, the marker signal and the dig signal is comparable. You may need to find out the amount of marker that you have to load per lane to be detected by the chromogenic substrate method.

Your probe with DIG amount may be much larger when compared with the DIG-labeled markers that are mixed with non-DIG labeled markers (not 100% of marker bands are labeled with DIG- only a small amount mixed in). We have a feeling that a separate protocol should be developed for your chromogenic substrate method. We believe the molar amount of DIG labeled probe is much larger, so the signal is very strong and the marker lanes do not have enough DIG to be detected by NBT/BCIP chromogenic method.